

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER <b>TAKAYAMA10</b>
<b>TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371</b>		U.S. APPLICATION NO (If known, see 37 CFR 1.5) <b>10/069481</b>
INTERNATIONAL APPLICATION NO. <b>PCT/JP00/05743</b>	INTERNATIONAL FILING DATE <b>25 AUGUST 2000</b>	PRIORITY CLAIMED <b>27 AUGUST 1999</b>
TITLE OF INVENTION <b>2<math>\alpha</math>-SUBSTITUTED VITAMIN D DERIVATIVES</b>		
APPLICANT(S) FOR DO/EO/US <b>Hiroaki TAKAYAMA et al.</b>		
<p>Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:</p> <ol style="list-style-type: none"> <li><input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li><input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li><input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).</li> <li><input checked="" type="checkbox"/> The US has been elected in a Demand by the expiration of 19 months from the priority date (PCT Article 31).</li> <li><input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2))             <ol style="list-style-type: none"> <li><input type="checkbox"/> is attached hereto (required only if not transmitted by the International Bureau).</li> <li><input checked="" type="checkbox"/> has been communicated by the International Bureau.</li> <li><input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</li> </ol> </li> <li><input checked="" type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).</li> <li><input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))             <ol style="list-style-type: none"> <li><input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau).</li> <li><input type="checkbox"/> have been communicated by the International Bureau.</li> <li><input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</li> <li><input checked="" type="checkbox"/> have not been made and will not be made.</li> </ol> </li> <li><input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</li> <li><input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).</li> <li><input type="checkbox"/> An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</li> </ol> <p><b>Items 11. to 16. below concern document(s) or information included:</b></p> <ol style="list-style-type: none"> <li><input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</li> <li><input type="checkbox"/> An Assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</li> <li><input checked="" type="checkbox"/> A <b>FIRST</b> preliminary amendment. <input type="checkbox"/> A <b>SECOND</b> or <b>SUBSEQUENT</b> preliminary amendment.</li> <li><input type="checkbox"/> A substitute specification.</li> <li><input type="checkbox"/> A change of power of attorney and/or address letter.</li> <li><input checked="" type="checkbox"/> Other items or information:             <ul style="list-style-type: none"> <li><input checked="" type="checkbox"/> Courtesy copy of the first page of the International Publication (WO 01/16099).</li> <li><input checked="" type="checkbox"/> Courtesy copy of the International Preliminary Examination Report. There were no annexes.</li> <li><input type="checkbox"/> Formal drawings, ____ sheets, Figures ____.</li> <li><input checked="" type="checkbox"/> Courtesy Copy of the International Search Report.</li> </ul> </li> </ol> <p><input checked="" type="checkbox"/> The application is (or will be) assigned to: CHUGAI SEIYAKU KABUSHIKI KAISHA, whose address is 5-1, Ukima 5-chome, Kita-ku, Tokyo 115-8543 Japan.</p>		

U.S. APPLICATION NO. (If known, see 37 CFR 1.6) <div style="font-size: 1.5em; font-weight: bold;">107069481</div>	International Application No. PCT/JP00/05743	Attorney's Docket No. TAKAYAMA10
--	---	-------------------------------------

17. [xx] The following fees are submitted: <b>BASIC NATIONAL FEE (37 CFR 1.492 (a)(1) - (5):</b> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO..... <b>\$1040.00</b>  International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO..... <b>\$890.00</b>  International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO..... <b>\$740.00</b>  International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)..... <b>\$710.00</b>  International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)..... <b>\$100.00</b>  <div style="text-align: center;"><b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b></div>	<div style="text-align: center; border-bottom: 1px solid black;"><b>CALCULATIONS PTO USE ONLY</b></div>
---	---

Surcharge of \$130.00 for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	\$	
Claims as Originally Presented	Number Filed	Number Extra
Total Claims	- 40	20
Independent Claims	- 3	0
Multiple Dependent Claims (if applicable)		+ \$280.00
<b>TOTAL OF ABOVE CALCULATIONS =</b>		<b>\$1,530.00</b>
Claims After Post Filing Prel. Amend	Number Filed	Number Extra
Total Claims	- 20	X \$18.00
Independent Claims	- 3	X \$84.00
<b>TOTAL OF ABOVE CALCULATIONS =</b>		<b>\$1,530.00</b>
Reduction of 1/2 for filing by small entity, if applicable. Applicant claims small entity status. See 37 CFR 1.27.		\$
<b>SUBTOTAL =</b>		<b>\$1,530.00</b>
Processing fee of \$130.00 for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).	\$	
<b>TOTAL NATIONAL FEE =</b>		<b>\$1,530.00</b>
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +	\$	
<b>TOTAL FEES ENCLOSED =</b>		<b>\$1,530.00</b>
		Amount to be: \$
		refunded
		charged \$

a. [ ] A check in the amount of \$\_\_\_\_\_ to cover the above fees is enclosed.

b. [xx] Credit Card Payment Form (PTO-2038), authorizing payment in the amount of \$ 1,530.00, is attached.

c. [ ] Please charge my Deposit Account No. **02-4035** in the amount of \$\_\_\_\_\_ to cover the above fees.  
A duplicate copy of this sheet is enclosed.

d. [XX] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **02-4035**. A duplicate copy of this sheet is enclosed.

**NOTE:** Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

**BROWDY AND NEIMARK, P.L.L.C.**  
**624 NINTH STREET, N.W., SUITE 300**  
**WASHINGTON, D.C. 20001**  
**TEL: (202) 628-5197**  
**FAX: (202) 737-3528**  
**Date of this submission: February 27, 2002**

SIGNATURE  
 Roger L. Browdy  
 NAME  
25,618  
 REGISTRATION NUMBER

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Claims 5 and 7 have been amended as follows:

5 (Amended). The vitamin D derivative according to one of claims 1 to 43, wherein R<sup>1</sup> is a 4-hydroxy-4-methylpentyl group.

7 (Amended). A pharmaceutical composition comprising a vitamin D derivative according to any one of claims 1, 2, 3 ~~to~~ or 6 as an active ingredient.

New claims 9-15 have been added.

2004 FEB 27 10 06 94 81

JC19 Rec'd PCT/PTO 27 FEB 2002

ATTY DOCKET: TAKAYAMA=10

Art Unit:

Washington, D.C.

February 27, 2002

February 27, 2002

February 27, 2002

February 27, 2002

February 27, 2002

Honorable Commissioner for Patents and Trademarks  
Washington, D.C. 20231

Sir:

Prior to examination on the merits, kindly amend as follows:

Please rewrite claims 5 and 7 in amended form as follows:

5 (Amended). The vitamin D derivative according to one of claims 1 to 3, wherein R<sup>1</sup> is a 4-hydroxy-4-methylpentyl group.

7 (Amended). A pharmaceutical composition comprising a vitamin D derivative according to any one of claims 1, 2, 3 or 6 as an active ingredient.

Please add new claims 9- as follows:

9 (New). The vitamin D derivative according to claim 4, wherein R<sup>1</sup> is a 4-hydroxy-4-methylpentyl group.

10 (New). A pharmaceutical composition comprising a vitamin D derivative according to claim 4 as an active ingredient.

11 (New). A pharmaceutical composition comprising a vitamin D derivative according to claim 5 as an active ingredient.

12 (New). A pharmaceutical composition comprising a vitamin D derivative according to claim 9 as an active ingredient.

13 (New). The pharmaceutical composition according to claim 10, wherein the composition is a therapeutic agent for a disease associated with abnormal calcium metabolism, an antitumor agent or an immunomodulator.

14 (New). The pharmaceutical composition according to claim 11, wherein the composition is a therapeutic agent for a disease associated with abnormal calcium metabolism, an antitumor agent or an immunomodulator.

15 (New). The pharmaceutical composition according to claim 12, wherein the composition is a therapeutic agent for a disease associated with abnormal calcium metabolism, an antitumor agent or an immunomodulator.

In re of: National Stage of PCT/JP00/05743

REMARKS

Claims 1-15 presently appear in this application.  
The present amendments have been made to place the case in  
better condition for examination.

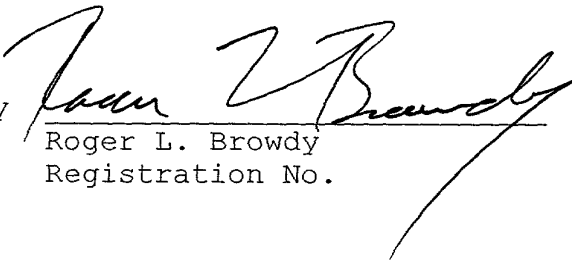
Attached hereto is a marked-up version of the  
changes made to the claims by the current amendment. The  
attached page is captioned "Version with Markings to Show  
Changes Made."

Favorable consideration and allowance are earnestly  
solicited.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.  
Attorneys for Applicant(s)

By

  
Roger L. Browdy  
Registration No.

RLB:rd  
Telephone No.: (202) 628-5197  
Facsimile No.: (202) 737-3528  
F:\,Y\YUAS\Takayama10\PTO\Preliminary Amendment.doc

## SPECIFICATION

2 $\alpha$ -SUBSTITUTED VITAMIN D DERIVATIVES5 TECHNICAL FIELD

The present invention relates to novel vitamin D derivatives, more particularly, relates to vitamin D derivatives having a substituent at the 2 $\alpha$ -position.

10 BACKGROUND ART

Active vitamins D<sub>3</sub> including 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> are known to exhibit a variety of physiological activities such as calcium metabolism regulatory activities, growth inhibitory and differentiation toward tumor cells, and immunoregulatory activities. However, some active vitamins D<sub>3</sub> disadvantageously may cause hypercalcemia during long-term and continuous administration, and are thus not suitable for use as antitumor agents, antirheumatic agents, or the like. Thus, a number of synthetic studies have been conducted for the purpose of obtaining vitamin D derivatives which are superior in certain activities.

The studies conducted by the inventors of the present invention revealed that 2 $\alpha$ -methyl derivatives of active vitamin D<sub>3</sub>, i.e., 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub>, increases binding property to vitamin D receptor (VDR) (K.Konno, et al., Bioorg. Med. Chem. Lett., 1998, 8, 151). Furthermore, a combination of the 2 $\alpha$ -methylation and epimerization at the 20-position on the side chain has been reported to

additionally enhance the binding property to VDR  
(T.Fujishima et al., Bioorg. Med. Chem. Lett., 1998, 8,  
2145).

However, no work has been done to synthesize a  
vitamin D derivative, in which the 2-position is  
substituted, other than with a methyl group, and the groups  
at the 20-position are epimerized; further, the  
physiological activities of such a vitamin D derivative  
have not been studied.

#### DISCLOSURE OF THE INVENTION

An object of the present invention is to synthesize  
and provide a novel vitamin D<sub>3</sub> derivative, in which the  
2-position is substituted, other than with a methyl group,  
the hydroxy groups at 1- and 3-positions are  $\alpha$ - and  $\beta$ -  
oriented, respectively, and the groups at the 20-position  
are epimerized.

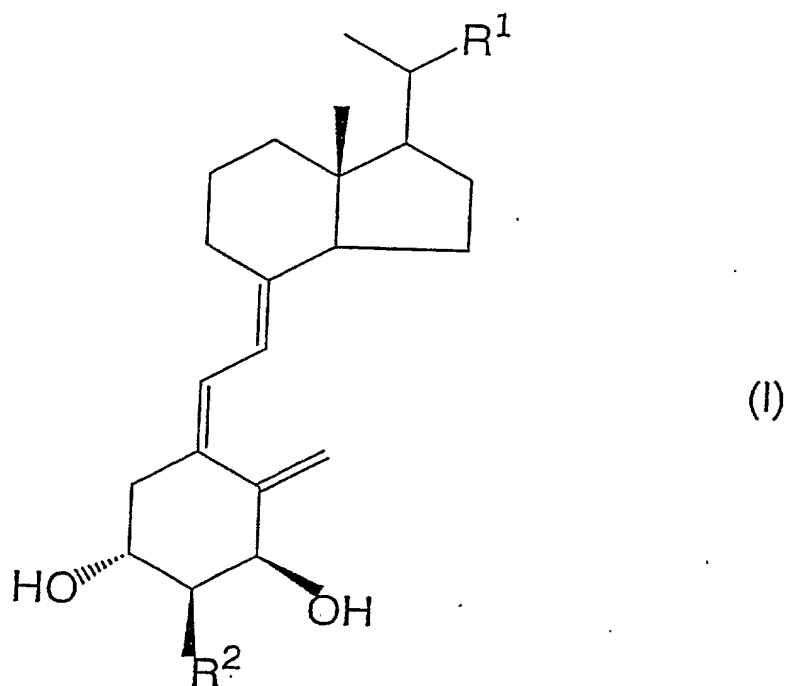
Another object of the present invention is to  
evaluate bioavailability of the synthesized vitamin D<sub>3</sub>  
derivative.

As a result of careful studies to achieve the above  
objects, the inventors of the present invention have  
succeeded in synthesizing vitamin D derivatives having a  
variety of configurations by synthesizing CD-ring compounds  
each having a desired side chain portion and then coupling  
it with A-ring compounds each having a desired group at the  
2 $\alpha$ -position, thereby completing the present invention.

According to an aspect of the present invention, a



vitamin D derivative represented by Formula (I) is provided:



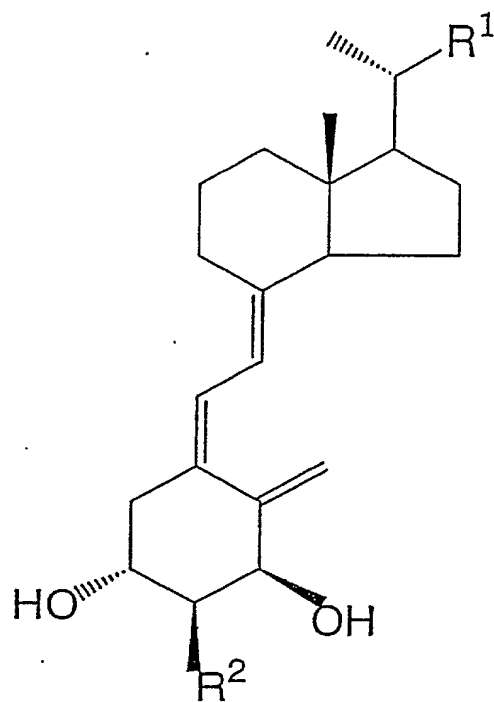
wherein

$R^1$  represents a saturated aliphatic  $C_{1-15}$ hydrocarbon group optionally substituted with 1 to 3 hydroxy or protected hydroxy groups; and

$R^2$  represents a saturated aliphatic  $C_{1-10}$ hydrocarbon group optionally substituted with one or more substituents, which may be the same or different and which are selected from the group consisting of a hydroxy group, a halogen atom, a cyano group, a lower alkoxy group, an amino group and an acylamino group, provided that when  $R^2$  represents a saturated aliphatic  $C_1$ hydrocarbon group,  $R^2$  is substituted with at least one substituent.

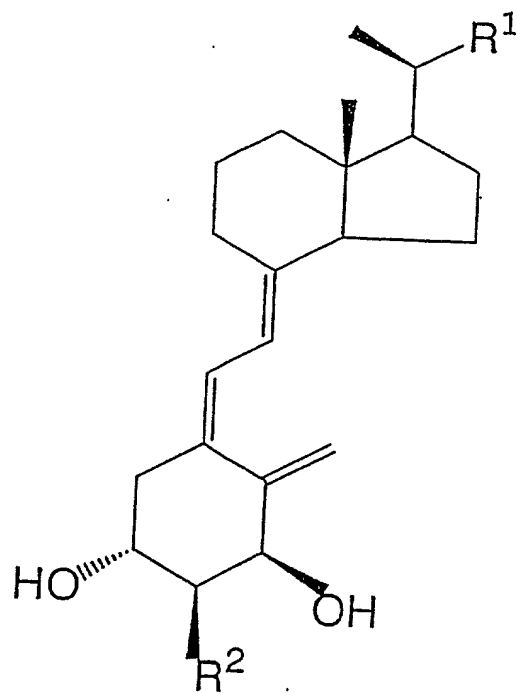
Among such vitamin D derivatives, those represented

by the following Formula (II) or



(II)

(III) are preferred.



(III)

In Formulae (I), (II) and (III), R<sup>2</sup> is preferably a hydroxymethyl, hydroxyethyl, hydroxypropyl, hydroxybutyl, hydroxypentyl, hydroxyhexyl, ethyl, propyl, butyl, pentyl or hexyl group.

5 In Formulae (I), (II) and (III), R<sup>1</sup> is preferably a saturated aliphatic C<sub>1-15</sub> hydrocarbon group substituted with one hydroxy or protected hydroxy group, more preferably R<sup>1</sup> is a 4-hydroxy-4-methylpentyl group.

Particularly preferred compounds of the present  
10 invention are those selected from the group consisting of (5Z,7E)-(1S,2S,3R,20R)-9,10-seco-5,7,10(19)-cholestatriene-2-hydroxymethyl-1,3,25-triol, (5Z,7E)-(1S,2S,3R,20R)-9,10-seco-5,7,10(19)-cholestatriene-2-(2'-hydroxyethyl)-1,3,25-triol, (5Z,7E)-(1S,2S,3R,20R)-9,10-seco-5,7,10(19)-  
15 cholestatriene-2-(3'-hydroxypropyl)-1,3,25-triol, (5Z,7E)-(1S,2S,3R,20R)-9,10-seco-5,7,10(19)-cholestatriene-2-(4'-hydroxybutyl)-1,3,25-triol, (5Z,7E)-(1S,2S,3R,20R)-9,10-seco-5,7,10(19)-cholestatriene-2-(5'-hydroxypentyl)-1,3,25-triol, (5Z,7E)-(1S,2S,3R,20R)-9,10-seco-5,7,10(19)-  
20 cholestatriene-2-(6'-hydroxyhexyl)-1,3,25-triol, (5Z,7E)-(1S,2S,3R,20R)-9,10-seco-5,7,10(19)-cholestatriene-2-ethyl-1,3,25-triol, (5Z,7E)-(1S,2S,3R,20R)-9,10-seco-5,7,10(19)-cholestatriene-2-propyl-1,3,25-triol, (5Z,7E)-(1S,2S,3R,20R)-9,10-seco-5,7,10(19)-cholestatriene-2-butyl-  
25 1,3,25-triol, (5Z,7E)-(1S,2S,3R,20R)-9,10-seco-5,7,10(19)-cholestatriene-2-pentyl-1,3,25-triol and (5Z,7E)-(1S,2S,3R,20R)-9,10-seco-5,7,10(19)-cholestatriene-2-hexyl-1,3,25-triol.

According to the present invention, a pharmaceutical composition comprising the above mentioned vitamin D derivative as an active ingredient is provided. Examples of such medicaments include therapeutic agents for diseases accompanied with abnormal calcium metabolism, an antitumor agent, an immunomodulator, and the like.

According to the present invention, use of the above mentioned vitamin D derivative for a medicament is provided. Examples of such medicaments include therapeutic agents for diseases accompanied with abnormal calcium metabolism, an antitumor agent, an immunomodulator, and the like.

#### PREFERRED MODE FOR CARRYING OUT THE INVENTION

Detailed modes and methods with respect to vitamin D derivatives represented by Formula (I) and pharmaceutical compositions including the vitamin D derivatives in accordance with the present invention are described in further detail below.

The contents of the specification of Japanese Patent Application No.11-241650, the application on the basis of which the present application claims priority are to be incorporated in their entirety by reference.

In Formula (I),  $R^1$  represents a saturated aliphatic  $C_{1-15}$  hydrocarbon group which may be substituted with 1 to 3 hydroxy groups or protected hydroxy groups.

In the present application, a saturated aliphatic hydrocarbon group generally means a straight or branched

alkyl group. Specific examples thereof include methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, i-butyl and tert-butyl groups, and further include pentyl, hexyl, heptyl, octyl, nonyl and decanyl groups. Among them, 3-methylbutyl, 3-ethylpentyl, 4-methylpentyl, 3-(n-propyl)hexyl, 4-ethylhexyl, 5-methylhexyl, 6-methylheptyl, 5-ethylheptyl and 4-(n-propyl)hepty groups are preferred.

$R^1$  is preferably a 5-methylhexyl, 4-ethylhexyl or 4-methylpentyl group.

The saturated aliphatic hydrocarbon groups optionally substituted with hydroxy groups means that any hydrogen atoms in the above mentioned saturated hydrocarbon groups may be substituted with one or more hydroxy groups.

In the definition of  $R^1$ , the number of hydroxy substituents is 0, 1, 2 or 3, preferably 1 or 2, and more preferably 1. Specific examples of the saturated aliphatic hydrocarbon groups substituted with at least one hydroxy group include 2-hydroxy-2-methylpropyl, 3-hydroxy-2-methylpropyl, 2,3-dihydroxy-2-methylpropyl, 2-ethyl-2-hydroxybutyl, 2-ethyl-3-hydroxybutyl, 2-ethyl-2,3-dihydroxybutyl, 2-hydroxy-2-(n-propyl)pentyl, 3-hydroxy-2-(n-propyl)pentyl, 2,3-dihydroxy-2-(n-propyl)pentyl, 2-hydroxy-3-methylbutyl, 3-hydroxy-3-methylbutyl, 4-hydroxy-3-methylbutyl, 2,3-dihydroxy-3-methylbutyl, 2,4-dihydroxy-3-methylbutyl, 3,4-dihydroxy-3-methylbutyl, 3-ethyl-2-hydroxypentyl, 3-ethyl-3-hydroxypentyl, 3-ethyl-4-hydroxypentyl, 3-ethyl-2,3-dihydroxypentyl, 3-ethyl-2,4-dihydroxypentyl, 3-ethyl-3,4-dihydroxypentyl, 2-hydroxy-3-

(n-propyl)hexyl, 3-hydroxy-3-(n-propyl)hexyl, 4-hydroxy-3-(n-propyl)hexyl, 2,3-dihydroxy-3-(n-propyl)hexyl, 2,4-dihydroxy-3-(n-propyl)hexyl, 3,4-dihydroxy-3-(n-propyl)hexyl, 3-hydroxy-4-methylpentyl, 4-hydroxy-4-methylpentyl, 5-hydroxy-4-methylpentyl, 3,4-dihydroxy-4-methylpentyl, 3,5-dihydroxy-4-methylpentyl, 4,5-dihydroxy-4-methylpentyl, 4-ethyl-3-hydroxyhexyl, 4-ethyl-4-hydroxyhexyl, 4-ethyl-5-hydroxyhexyl, 4-ethyl-3,4-dihydroxyhexyl, 4-ethyl-3,5-dihydroxyhexyl, 4-ethyl-4,5-dihydroxyhexyl, 3-hydroxy-4-(n-propyl)heptyl, 4-hydroxy-4-(n-propyl)heptyl, 5-hydroxy-4-(n-propyl)heptyl, 3,4-dihydroxy-4-(n-propyl)heptyl, 3,5-dihydroxy-4-(n-propyl)heptyl, 4,5-dihydroxy-4-(n-propyl)heptyl, 4-hydroxy-5-methylhexyl, 5-hydroxy-5-methylhexyl, 6-hydroxy-5-methylhexyl, 4,5-dihydroxy-5-methylhexyl, 4,6-dihydroxy-5-methylhexyl, 5,6-dihydroxy-5-methylhexyl, 5-ethyl-4-hydroxyheptyl, 5-ethyl-5-hydroxyheptyl, 5-ethyl-6-hydroxyheptyl, 5-ethyl-4,5-dihydroxyheptyl, 5-ethyl-4,6-dihydroxyheptyl, 5-ethyl-5,6-dihydroxyheptyl, 4-hydroxy-5-(n-propyl)octyl, 5-hydroxy-5-(n-propyl)octyl, 6-hydroxy-5-(n-propyl)octyl, 4,5-dihydroxy-5-(n-propyl)octyl, 4,6-dihydroxy-5-(n-propyl)octyl, 5,6-dihydroxy-5-(n-propyl)octyl, 5-hydroxy-6-methylheptyl, 6-hydroxy-6-methylheptyl, 7-hydroxy-6-methylheptyl, 5,6-dihydroxy-6-methylheptyl, 5,7-dihydroxy-6-methylheptyl, 6,7-dihydroxy-6-methylheptyl, 6-ethyl-5-hydroxyoctyl, 6-ethyl-6-hydroxyoctyl, 6-ethyl-7-hydroxyoctyl, 6-ethyl-5,6-dihydroxyoctyl, 6-ethyl-5,7-dihydroxyoctyl, 6-ethyl-6,7-

10055431.022702  
20220724 14:59:07

dihydroxyoctyl, 5-hydroxy-6-(n-propyl)nonyl, 6-hydroxy-6-(n-propyl)nonyl, 7-hydroxy-6-(n-propyl)nonyl, 5,6-dihydroxy-6-(n-propyl)nonyl, 5,7-dihydroxy-6-(n-propyl)nonyl and 6,7-dihydroxy-6-(n-propyl) groups. Among them, 4-hydroxy-4-methylpentyl, 4-ethyl-4-hydroxyhexyl, 5-hydroxy-5-methylhexyl and 5-ethyl-5-hydroxyheptyl groups are more preferred and a 4-hydroxy-4-methylpentyl group is further more preferred.

Examples of protecting groups for the hydroxy groups in R<sup>1</sup> of Formulae (I), (II) and (III) include acyl groups, substituted silyl groups and substituted alkyl groups, preferably acyl groups and substituted silyl groups.

Acyl groups mean substituted carbonyl groups; examples of the substituents of the carbonyl groups include a hydrogen atom, optionally substituted lower alkyl groups, optionally substituted aryl groups, optionally substituted lower alkyloxy groups, optionally substituted aryloxy groups, optionally substituted aralkyloxy groups and the like. Preferred examples of the acyl groups include a formyl group, lower alkylcarbonyl groups, optionally substituted phenylcarbonyl groups, lower alkyloxycarbonyl groups, optionally substituted phenylalkyloxycarbonyl groups and the like, more preferred examples include formyl, acetyl, propionyl, butyryl, pivaloyl, benzoyl, methoxycarbonyl, ethoxycarbonyl, tert-butoxycarbonyl and benzyloxycarbonyl groups.

Substituted silyl groups mean silyl groups substituted with lower alkyl groups which may have one or

more substituents, optionally substituted aryl groups and the like. Preferably, substituted silyl groups mean tri-substituted silyl groups. Preferred examples of the substituted silyl groups include trimethylsilyl, triethylsilyl, triisopropylsilyl, tert-butyldiphenylsilyl and tert-butyldimethylsilyl groups.

Substituted alkyl groups mean those alkyl groups which are substituted with one or more substituents. Preferred examples of the substituents include optionally substituted alkyloxy groups and optionally substituted aryl groups, with optionally substituted alkyloxy groups being particularly preferred. Examples of the alkyl groups substituted with an optionally substituted alkyloxy group (such as an alkyloxy group) include, for example, methoxymethyl, 2-methoxyethoxymethyl and tetrahydropyran-2-yl groups. Examples of the substituents include halogen atoms and cyano, nitro, amino, hydroxy, alkyl, alkyloxy, acyloxy and sulfonyl groups and the like.

In Formulae (I), (II) and (III),  $R^2$  represents a saturated aliphatic hydrocarbon group which may be substituted with one or more of substituents selected from the group consisting of a hydroxy group, halogen atoms, cyano, lower alkoxy, amino and acylamino groups.

Examples of the saturated aliphatic hydrocarbon groups include the above described straight or branched alkyl groups, preferably having 1-10 carbon atoms, more preferably 1-6 carbon atoms, and particularly preferably 3-5 carbon atoms.



The halogen atoms include fluorine, chlorine, bromine and iodine atoms, and the lower alkoxy groups mean those having 1-6 carbon atoms. The hydroxy and amino group may have protecting groups.

5        Similar to the above, preferred examples of the acyl groups of the acylamino group include a formyl group, lower alkylcarbonyl groups, optionally substituted phenylcarbonyl groups, lower alkyloxycarbonyl groups, optionally substituted phenylalkyloxycarbonyl groups and the like,  
10       more preferably formyl, acetyl, propionyl, butyryl, pivaloyl, benzoyl, methoxycarbonyl, ethoxycarbonyl, tert-butoxycarbonyl and benzyloxycarbonyl groups.

As the substituents, a hydroxy group and a halogen atom are preferred. The number of substituents is 0, 1, 2  
15       or 3, preferably 1 or 2, and more preferably 1.

It is particularly preferred that  $R^2$  is a hydroxymethyl, hydroxyethyl, hydroxypropyl, hydroxybutyl, hydroxypentyl, hydroxyhexyl, ethyl, propyl, butyl, pentyl or hexyl group.

20       The stereochemistry of the hydroxy groups at the 1- and 3-positions and the methyl group at the 2-position in compounds of Formula (I) of the present invention can occur in  $\alpha$  or  $\beta$  conformation, and the respective compounds are all included within the scope of the present invention.

25       Examples of the compounds of Formula (I) of the present invention include  $1\alpha, 25$ -dihydroxy- $2\alpha$ -hydroxymethylvitamin  $D_3$ ,  $1\alpha, 25$ -dihydroxy- $2\alpha$ -hydroxyethylvitamin  $D_3$ ,  $1\alpha, 25$ -dihydroxy- $2\alpha$ -

hydroxypropylvitamin D<sub>3</sub>, 1 $\alpha$ ,25-dihydroxy-2 $\alpha$ -  
 hydroxybutylvitamin D<sub>3</sub>, 1 $\alpha$ ,25-dihydroxy-2 $\alpha$ -  
 hydroxypentylvitamin D<sub>3</sub>, 1 $\alpha$ ,25-dihydroxy-2 $\alpha$ -  
 hydroxyhexylvitamin D<sub>3</sub>, 1 $\alpha$ ,25-dihydroxy-2 $\alpha$ -ethylvitamin D<sub>3</sub>,  
 5 1 $\alpha$ ,25-dihydroxy-2 $\alpha$ -propylvitamin D<sub>3</sub>, 1 $\alpha$ ,25-dihydroxy-2 $\alpha$ -  
 butylvitamin D<sub>3</sub>, 1 $\alpha$ ,25-dihydroxy-2 $\alpha$ -pentylvitamin D<sub>3</sub> and  
 1 $\alpha$ ,25-dihydroxy-2 $\alpha$ -hexylvitamin D<sub>3</sub>. Particularly preferred  
 examples include (5Z,7E)-(1S,2S,3R,20R)-9,10-seco-  
 5,7,10(19)-cholestatriene-2-hydroxymethyl-1,3,25-triol,  
 10 (5Z,7E)-(1S,2S,3R,20R)-9,10-seco-5,7,10(19)-cholestatriene-  
 2-(2'-hydroxyethyl)-1,3,25-triol, (5Z,7E)-(1S,2S,3R,20R)-  
 9,10-seco-5,7,10(19)-cholestatriene-2-(3'-hydroxypropyl)-  
 1,3,25-triol, (5Z,7E)-(1S,2S,3R,20R)-9,10-seco-5,7,10(19)-  
 cholestatriene-2-(4'-hydroxybutyl)-1,3,25-triol, (5Z,7E)-  
 15 (1S,2S,3R,20R)-9,10-seco-5,7,10(19)-cholestatriene-2-(5'-  
 hydroxypentyl)-1,3,25-triol, (5Z,7E)-(1S,2S,3R,20R)-9,10-  
 seco-5,7,10(19)-cholestatriene-2-(6'-hydroxyhexyl)-1,3,25-  
 triol, (5Z,7E)-(1S,2S,3R,20R)-9,10-seco-5,7,10(19)-  
 cholestatriene-2-ethyl-1,3,25-triol, (5Z,7E)-  
 20 (1S,2S,3R,20R)-9,10-seco-5,7,10(19)-cholestatriene-2-  
 propyl-1,3,25-triol, (5Z,7E)-(1S,2S,3R,20R)-9,10-seco-  
 5,7,10(19)-cholestatriene-2-butyl-1,3,25-triol, (5Z,7E)-  
 (1S,2S,3R,20R)-9,10-seco-5,7,10(19)-cholestatriene-2-  
 pentyl-1,3,25-triol and (5Z,7E)-(1S,2S,3R,20R)-9,10-seco-  
 25 5,7,10(19)-cholestatriene-2-hexyl-1,3,25-triol.

Although there is no limitation with respect to  
 methods for synthesizing the compounds represented by  
 Formula (I) of the present invention, for example, A-ring

and CD-ring parts of a vitamin D derivative are separately synthesized and then subjected to coupling, as described in the following Examples.

CD-ring part compounds of the vitamin D derivatives  
5 are known. A desired CD-ring compound can be obtained by appropriately modifying a side chain of a known CD-ring compound or can be obtained from a known CD-ring compound having a corresponding side chain.

These known vitamin D derivatives are disclosed in  
10 Japanese Patent Publication (Kokai) Nos. 61-267550, 6-72994 and 6-256300, Japanese Patent Publication (Kohyo) Nos. 4-503669 and 4-504573, and Japanese Patent Publication (Kokai) No. 10-182597, WO 94/14766, WO 95/27697, and the like. After protecting the hydroxy group of the above  
15 mentioned vitamin D derivative with a protecting group, the resultant compound is subjected to ozonolysis, and then, to  $\text{NaBH}_4$  reduction, giving an alcohol having a hydroxy group at the 8-position. Oxidization using an appropriate oxidant gives a ketone having an oxo group at the 8-  
20 position. A CD-ring compound having a desired side chain can be obtained by converting the oxo group at the 8-position with a bromomethylene group.

A-ring compounds having a substituent at the  $2\beta$ -  
position are known and are described by K. Konno et al.  
25 (Bioorg. Med. Chem. Lett., 8(1998) P.151-156) and T. Fujishima et al. (Bioorg. Med. Chem. Lett., 8(1998), P.2145-2148).

A-ring compounds having a substituent at the  $2\alpha$ -

position can be synthesized from a starting material, such as 1,2-O-isopropylidene- $\alpha$ -D-(R)-xylofuranose, according to the process described in the following Examples, however there is no limitation with respect to a method for synthesizing the compounds. Compounds other than those described in the following Examples can also be synthesized in the same manner by using corresponding starting materials.

Coupling of an A-ring compound with a CD-ring compound can be carried out using a known method. An A-ring compound, which is obtainable by the above method and which has a triple bond at one terminal and a double bond at the other terminal, is reacted with a CD-ring compound, which has a bromomethylene group at the coupling site for the A-ring compound, in the presence of a palladium catalyst such as  $\text{Pd}_2(\text{dba})_3$  and triphenylphosphine ( $\text{PPh}_3$ ) in an appropriate solvent.

After the coupling reaction, the resulting product is purified in a usual manner such as thin layer chromatography and subjected to removal of the hydroxy protecting group, to give a desired vitamin D derivative having a substituent at the  $2\alpha$ -position.

The compounds of the present invention are preferably formulated into appropriate dosage forms with pharmaceutically acceptable carriers, excipients, disintegrants, lubricants, binders, flavors, colorants and the like; examples of the dosage forms include tablets, granules, fine granules, capsules, powders, injections,

20220104 100654B1 022702

solutions, suspensions, emulsions, percutaneous administration formulations, suppositories and the like.

There is no restriction on routes of administration for the compounds of the present invention; the compounds  
5 may be administered orally or parenterally (intravenously, intramuscularly, intraperitoneally, percutaneously and the like).

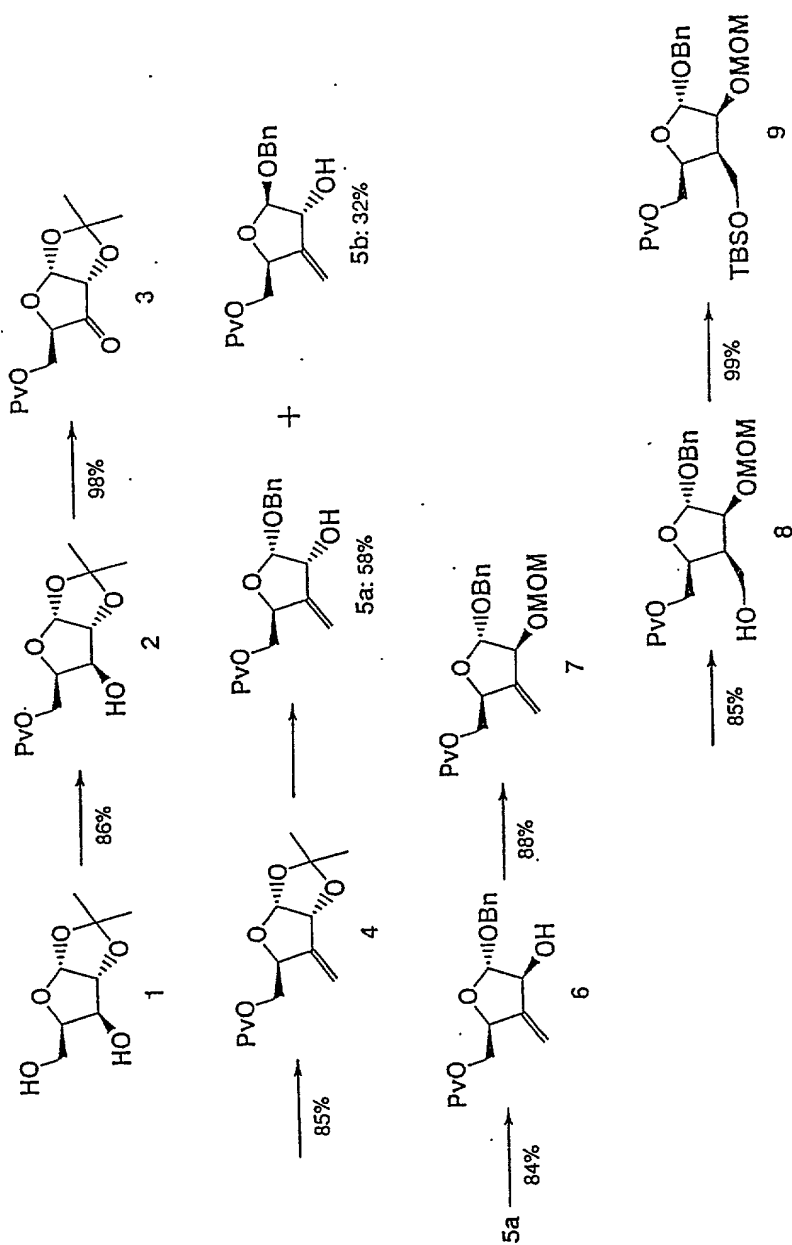
Dosage of compounds of the present invention can be appropriately chosen depending on target disease,  
10 conditions, body type, constitution, age and sex of the patient, administration route, dosage form and other factors. Typically, the lower limit for an adult ranges from 0.001  $\mu\text{g}$  to 0.1  $\mu\text{g}$  and preferably around 0.01  $\mu\text{g}$  daily, and the upper limit for an adult ranges from 100 $\mu\text{g}$   
15 to 10000  $\mu\text{g}$  and preferably from 200  $\mu\text{g}$  to 1000  $\mu\text{g}$  daily, which may be administered at a time or in divided portions twice or three times a day.

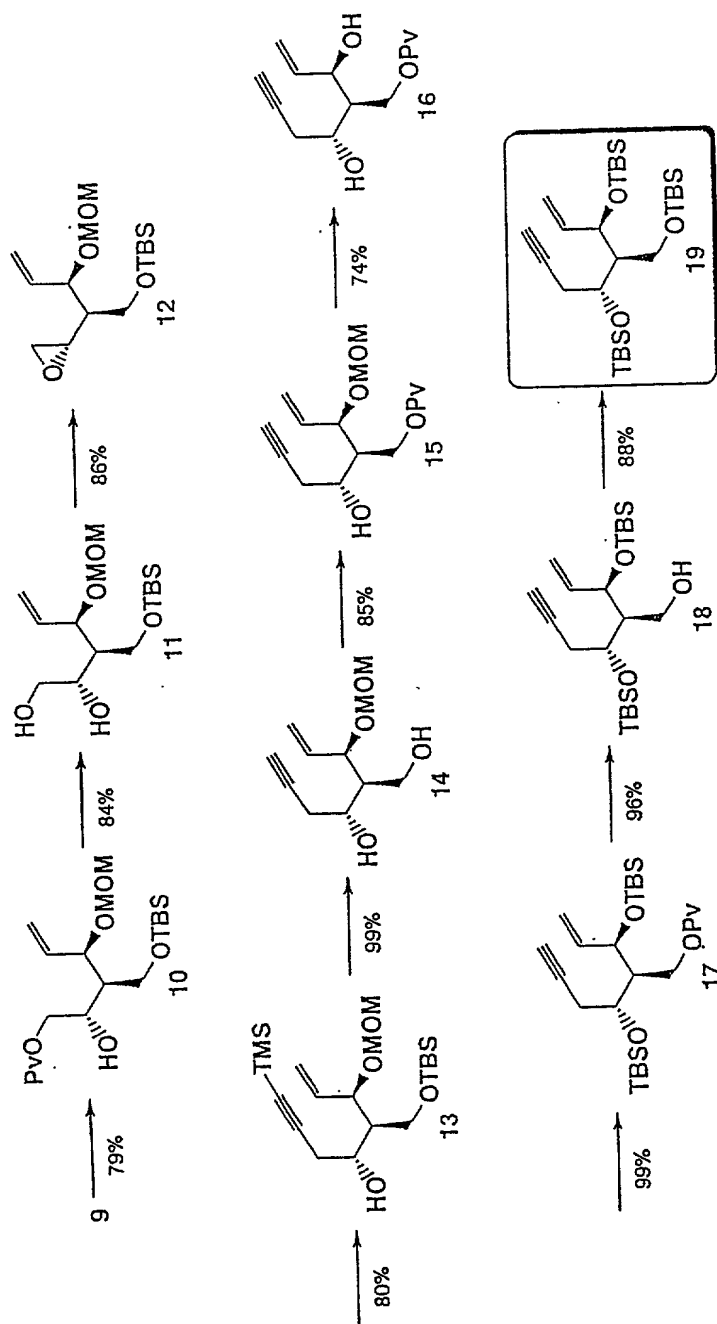
#### EXAMPLES

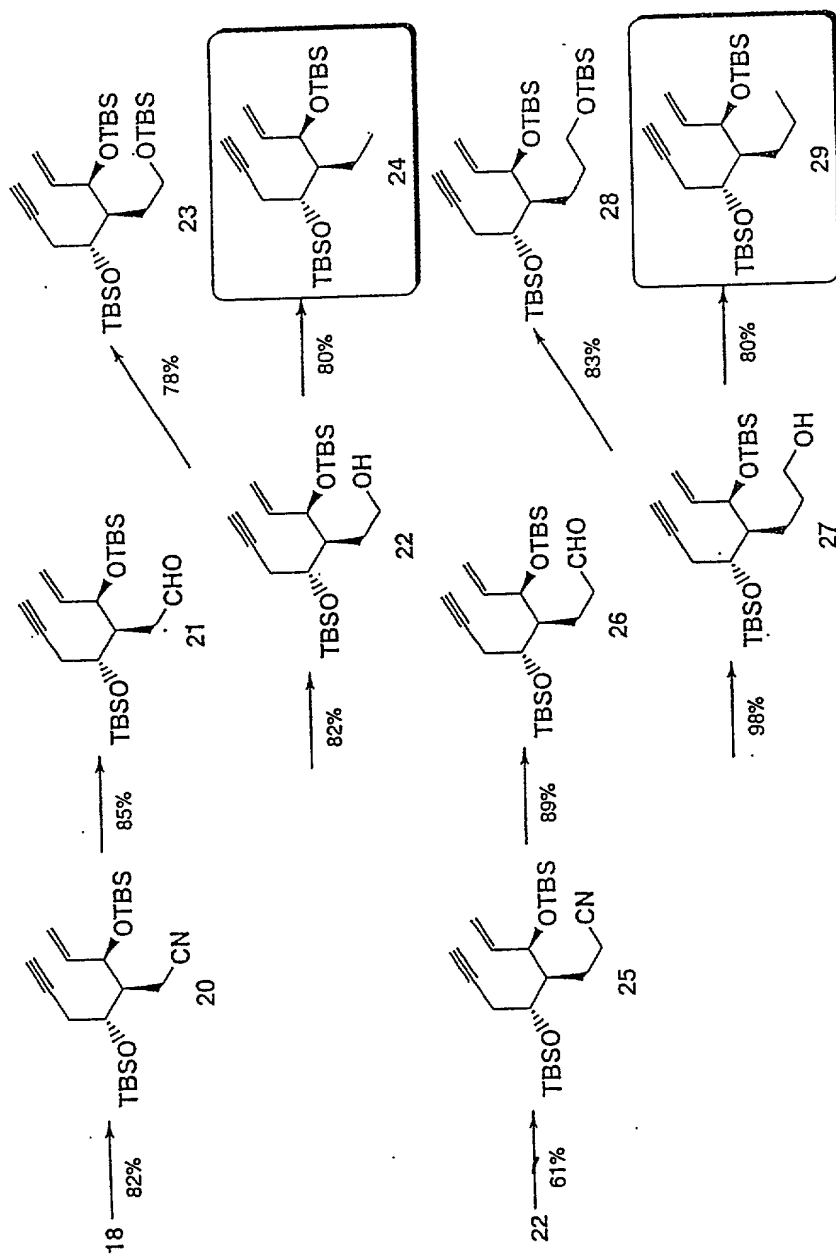
20 The present invention will be described specifically by way of the following Examples, which in no way limit the invention.

(Example 1): Synthesis of an A-ring compound used for the synthesis of a vitamin D derivative having a substituent at  
25 the 2 $\alpha$ -position.

Example 1 was conducted according to the following reaction scheme:









(1) Synthesis of 5-O-pivaloyl-1,2-O-isopropylidene- $\alpha$ -D-xylofuranose (Compound 2)

1,2-O-isopropylidene- $\alpha$ -D-xylofuranose (Compound 1, 15.0 g, 78.9 mmol) was dissolved in pyridine (70 mL), cooled to 0°C under argon atmosphere, and trimethylacetyl chloride (9.9 g, 82.2 mmol) was added dropwise over 2 hours. The reaction mixture was stirred for 10 hours at that temperature, then MeOH (5 mL) was added and the mixture was concentrated. The residue was dissolved in diethyl ether (500 mL) and washed with water (100 mL), saturated aqueous copper sulfate solution (100 mL), water (100 mL) and saturated brine (100 mL). The aqueous layer was extracted with 100 mL of diethyl ether three times. The ether layers were combined with the previously obtained diethyl ether layer. The ether layer thus obtained was dried over magnesium sulfate, filtered and concentrated. The residue was purified by silica gel chromatography (ethyl acetate/hexane 1:4) to give Compound 2 (18.7 g, 86%) as a colorless oil.

$[\alpha]^{20}_D$  2.69 (c 1.00,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.22 (s, 9H), 1.32 (s, 3H), 1.51 (s, 3H), 4.10 (d, 1H,  $J$  = 2.8 Hz), 3.78 (bs, 1H), 4.17 (dd, 1H,  $J$  = 5.6, 11.2 Hz), 4.25 (ddd, 1H,  $J$  = 2.8, 5.6, 7.2 Hz), 4.50 (dd, 1H,  $J$  = 7.2, 11.2 Hz), 4.56 (d, 1H,  $J$  = 3.6 Hz), 5.93 (d, 1H,  $J$  = 3.6 Hz);

HREIMS  $\text{C}_{12}\text{H}_{19}\text{O}_6$  ( $\text{M}^+ - \text{CH}_3$ ) calcd. 259.1182, found 259.1182.

(2) Synthesis of 5-O-pivaloyl-1,2-O-isopropylidene- $\alpha$ -D-erythro-3-pentofuranose (Compound 3)

Compound 2 (10.5 g, 38.3 mmol) was dissolved in

1065431 062702  
dichloromethane (800 mL), zeolite (75 g) and PCC  
(pyridinium chlorochromate) (36.5 g, 169.4 mmol) were added  
at room temperature and the mixture was stirred for 3  
hours. The reaction mixture was diluted with hexane (800  
5 mL), filtered and concentrated. The residue was purified  
by silica gel chromatography (ethyl acetate/hexane 2:3) to  
give ketone Compound 3 (10.2 g, 98%) as a colorless oil.  
[ $\alpha$ ]<sub>D</sub><sup>20</sup> 4.19 (c 1.13, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.18  
(s, 9H), 1.44 (s, 3H), 1.48 (s, 3H), 4.23 (d, 1H, J = 3.2,  
10 11.6 Hz), 4.37 (dd, 1H, J= 1.2, 3.2 Hz), 4.39 (dd, 1H, J=  
3.2, 11.6 Hz), 4.57 (dt, 1H, J= 1.2, 3.2 Hz), 6.10 (d, 1H,  
J= 4.4 Hz); HREIMS C<sub>13</sub>H<sub>20</sub>O<sub>6</sub> (M<sup>+</sup>) calcd. 276.1260, found  
276.1262.

(3) Synthesis of 5-O-pivaloyl-1,2-O-isopropylidene-3-deoxy-  
15 3-C-methylene- $\alpha$ -D-xylo-pentofuranose (Compound 4)

Methyltriphenylphosphonium bromide (7.7 g, 21.6 mmol)  
was mixed with THF (100 mL), and 1.0M NaHMDS (sodium  
bis(trimethylsilyl)amide) in THF (18.0 mL, 18.0 mmol) was  
added dropwise at room temperature. The yellow reaction  
20 suspension was stirred for 1.5 hours at room temperature,  
cooled to -78°C under argon atmosphere, and Compound 3 (4.3  
g, 15.8 mmol) was slowly added. The reaction mixture was  
stirred at that temperature for 30 min., then warmed to  
room temperature and stirred for further 1 hour. The  
25 reaction mixture was cooled to 0°C, then MeOH (10 mL) was  
added. The mixture was diluted with diethyl ether (200  
mL), and washed with a saturated aqueous ammonium chloride  
solution (100 mL) and with saturated brine (100 mL), each

three times. The aqueous layer was extracted with 100 mL of diethyl ether three times and combined with the previously obtained ether layer. The ether layer was dried over magnesium sulfate, filtered and concentrated. The residue was purified by flash column chromatography (ethyl acetate/hexane 1:9) to give olefin Compound 4 (3.6 g, 85%) as a colorless oil.

$[\alpha]^{20}_D$  13.45 (c 1.31,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.20 (s, 9H), 1.39 (s, 3H), 1.52 (s, 3H), 4.19 (dd, 1H,  $J = 4.8, 12.0$  Hz), 4.23 (dd, 1H,  $J = 3.6, 12.0$  Hz), 4.91 (dd, 1H,  $J = 1.2, 4.0$  Hz), 4.94 (td, 1H,  $J = 2.6, 3.4, 4.8$  Hz), 5.22 (t, 1H,  $J = 1.2$  Hz), 5.48 (dd, 1H,  $J = 1.2, 2.4$  Hz), 5.87 (d, 1H,  $J = 4.0$  Hz); HREIMS  $\text{C}_{13}\text{H}_{19}\text{O}_5$  ( $\text{M}^+ - \text{Me}$ ) calcd. 255.1235, found 255.1232.

(4) Synthesis of benzyl 5-O-pivaloyl-3-deoxy-3-C-methylene- $\alpha$ -D-xylo-pentofuranose (Compound 5a) and benzyl 5-O-pivaloyl-3-deoxy-3-C-methylene- $\beta$ -D-xylo-pentofuranose (Compound 5b)

Compound 4 (3.2 g, 11.9 mmol) and benzyl alcohol (8.0 g, 74.1 mmol) were dissolved in toluene (22 mL), cooled to  $0^\circ\text{C}$ , and 4.0M hydrogen chloride-dioxane solution (10 mL, 40.0 mmol) was added. The reaction mixture was warmed to room temperature, stirred for 16 hours and diluted with a diethyl ether (200 mL). The diluted solution was cooled to  $0^\circ\text{C}$  and neutralized with saturated aqueous sodium bicarbonate solution (100 mL). The organic layer was washed with water (50 mL) and with saturated brine (50 mL) each three times. The aqueous layer was extracted with

50 mL of diethyl ether three times, combined with the previously obtained ether layer, dried over magnesium sulfate, filtered and concentrated. The residue was purified by flash column chromatography (ethyl

5 acetate/hexane 1:4) to give Compound 5a (2.2 g, 58%) and Compound 5b (1.22 g, 32%) as colorless oily alcohols.

5a:  $[\alpha]^{20}_D$  19.56 (c 1.23,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.20 (s, 9H), 2.26 (d, 1H,  $J = 11.6$  Hz), 4.18 (dd, 1H,  $J = 4.8, 12.0$  Hz), 4.60 (d, 1H,  $J = 12.0$  Hz), 4.73 (ddt, 1H,  $J = 2.4, 3.4, 4.8$  Hz), 4.81 (d, 1H,  $J = 12.0$  Hz), 5.15 (d, 1H,  $J = 4.4$  Hz), 5.18 (t, 1H,  $J = 2.4$  Hz), 5.39 (t, 1H,  $J = 2.4$  Hz), 7.34 (m, 5H). 5b:  $[\alpha]^{20}_D$  -4.00 (c 1.07,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.27 (s, 9H), 2.61 (bs, 1H), 4.14 (dd, 1H,  $J = 6.8, 12.0$  Hz), 4.19 (dd, 1H,  $J = 4.8, 12.0$  Hz), 4.42 (t, 1H,  $J = 1.2$  Hz), 4.49 (d, 1H,  $J = 11.6$  Hz), 4.76 (d, 1H,  $J = 11.6$  Hz), 4.89 (qt, 1H,  $J = 1.2, 4.8, 6.2$  Hz), 5.03 (s, 1H), 5.26 (t, 1H,  $J = 1.2$  Hz), 7.33 (m, 5H).

(5) Synthesis of benzyl 5-O-pivaloyl-3-deoxy-3-C-methylene- $\alpha$ -D-xylo-pentofuranoside (Compound 6)

20 Compound 5a (2.0 g, 6.25 mmol), p-nitrobenzoic acid (2.1 g, 12.6 mmol) and triphenylphosphine (3.3 g, 12.6 mmol) were dissolved in THF (40 mL), cooled to  $0^\circ\text{C}$  under argon atmosphere, and a 40% DEAD-toluene solution (5.8 g, 13.9 mmol) was added. The reaction mixture was stirred at 25 that temperature for 15 min., warmed to room temperature and stirred for further 3 hours. The reaction mixture was cooled to  $0^\circ\text{C}$ , then MeOH (10 mL) was added. The mixture was diluted with diethyl ether (100 mL) and washed with

202207045001  
1069481.022702

saturated aqueous sodium bicarbonate solution (50 mL) and saturated brine (50 mL), each three times. The aqueous layer was extracted with 50 mL of diethyl ether three times, combined with the previously obtained ether layer.

5 The ether layer was dried over magnesium sulfate, filtered and concentrated. The residue was purified by flash column chromatography (ethyl acetate/hexane 1:10) to give a crude ester compound as a colorless oil, which was subjected to the next reaction without further purification.

10 The ester was dissolved in MeOH (100 mL), cooled to 0°C, and 1.0M aqueous sodium hydroxide solution (1.0 mL, 1.00 mmol) was added dropwise. The reaction mixture was stirred for 1 hour at the same temperature, neutralized by the addition of 1.0 M hydrochloric acid (1.5 mL, 1.5 mmol)

15 and concentrated. The residue was dissolved in diethyl ether (200 mL) and washed with a saturated aqueous sodium bicarbonate solution (50 mL) and with saturated brine (50 mL) each three times. The aqueous layer was extracted with 50 mL of diethyl ether three times, combined with the

20 previously obtained ether layer. The ether layer was dried over magnesium sulfate, filtered and concentrated. The residue was purified by silica gel chromatography (ethyl acetate/hexane 1:5) to give alcohol Compound 6 (1.67 g, 84%) as a colorless oil.

25  $[\alpha]^{20}_D$  11.18 (c 1.00,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.22 (s, 9H), 2.09 (d, 1H,  $J$  = 7.6 Hz), 4.25 (dd, 1H,  $J$  = 4.8, 12.4 Hz), 4.33 (dd, 1H,  $J$  = 3.2, 12.4 Hz), 4.37 (d, 1H,  $J$  = 7.6 Hz), 4.55 (d, 1H,  $J$  = 11.6 Hz), 4.73 (ddd, 1H,  $J$  = 1.6,

3.2, 4.3 Hz), 4.74 (d, 1H, J= 11.6 Hz), 5.10 (s, 1H), 5.26 (s, 1H), 5.54 (t, 1H, J= 1.6 Hz), 7.33 (m, 5H).

(6) Synthesis of benzyl 5-O-pivaloyl-2-methoxymethyl-3-deoxy-3-C-methylene- $\alpha$ -D-xylofuranose (Compound 7)

5 Compound 6 (1.0 g, 3.13 mmol) was dissolved in dichloromethane (25 mL), to which diisopropylethylamine (1.24 g, 9.61 mmol) and chloromethyl ethyl ether (1.29 g, 16.0 mmol) were added dropwise at 0°C. Tetrabutylammonium iodide (360 mg, 975  $\mu$ mol) was added to the reaction mixture  
10 and then the mixture was stirred for 14 hours at room temperature in a dark. After the completion of the reaction, the reaction mixture was diluted with diethyl ether (150 mL) and washed with saturated aqueous ammonium chloride solution (25 mL) and with saturated brine (25 mL),  
15 each three times. The organic layer was dried over magnesium sulfate, filtered and concentrated. The residue was purified by silica gel chromatography (ethyl acetate/hexane 1:9) to give Compound 7 (1.01 g, 88%) as a colorless oil.

20  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.23 (s, 9H), 3.35 (s, 3H), 4.18 (dd, 1H, J= 6.4, 11.2 Hz), 4.22 (dd, 1H, J= 5.2, 11.2 Hz), 4.37 (s, 1H), 4.55 (d, 1H, J= 12.0 Hz), 4.60 (d, 1H, J= 6.8 Hz), 4.69 (ddd, 1H, J= 1.6, 5.2, 6.4 Hz), 4.75 (d, 1H, J= 12.0 Hz), 4.76 (d, 1H, J= 6.8 Hz), 5.21 (s, 1H), 5.36 (s,  
25 1H), 5.46 (d, 1H, J= 1.6 Hz), 7.33 (m, 5H).

(7) Synthesis of benzyl 5-O-pivaloyl-2-methoxymethyl-3-deoxy-3-C-hydroxymethyl- $\alpha$ -D-xylo-pentofuranose (Compound 8)

Compound 7 (2.0 g, 5.49 mmol) was dissolved in THF

202201345001  
(20 mL), cooled to 0°C, and a THF solution of 0.5M 9-BBN  
(9-borabicyclo[3,3,1]nonane) (20 mL, 10.0 mmol) was added  
dropwise. The reaction mixture was heated to 50°C, stirred  
for 3 hours and cooled to 0°C. To the mixture, 3.0M sodium  
5 hydroxide solution (6.4 mL, 19.2 mmol) and 30% hydrogen  
peroxide solution (12.8 mL) were added, followed by  
stirring vigorously for 2 hours at room temperature. The  
mixture was diluted with ethyl acetate (200 mL) and washed  
with water (50 mL), 5% aqueous sodium sulfite solution (50  
10 mL) and with brine (50 mL), each three times. The thus  
obtained aqueous layer was extracted with 50 mL of diethyl  
ether three times. The ether layer was combined with the  
previously obtained organic layer, dried over magnesium  
sulfate, filtered and concentrated. The thus obtained  
15 residue was purified by silica gel chromatography (ethyl  
acetate/hexane 1:3) to give Compound 8 (1.78 g, 85%) as a  
colorless oil.

$[\alpha]_D^{20}$  7.06 (c 1.70,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.22  
(s, 9H), 2.34 (t, 1H,  $J = 6.0$  Hz), 2.89 (tt, 1H,  $J = 6.0, 8.4$   
20 Hz), 3.37 (s, 3H), 3.86 (dt, 1H,  $J = 6.0, 11.2$  Hz), 3.88  
(ddd, 1H,  $J = 6.0, 8.4, 11.2$  Hz), 4.19 (dd, 1H,  $J = 7.2, 11.6$   
Hz), 4.20 (d, 1H,  $J = 6.0$  Hz), 4.23 (dd, 1H,  $J = 5.6, 11.6$   
Hz), 4.38 (dd, 1H,  $J = 5.6, 7.2, 8.4$  Hz), 4.50 (d, 1H,  $J =$   
12.0 Hz), 4.66 (s, 2H), 4.73 (d, 1H,  $J = 12.0$  Hz), 5.15 (s,  
25 1H), 7.34 (m, 5H).

(8) Synthesis of benzyl 5-O-pivaloyl-2-methoxymethyl-3-  
deoxy-3-C-(tert-butyldimethylsilyloxymethyl)- $\alpha$ -D-xylo-  
pentofuranose (Compound 9)

Compound 8 (2.97 g, 7.77 mmol) was dissolved in DMF (30 mL), to which solution imidazole (1.06 g, 15.6 mmol) and TBDMSCl (tert-butyldimethylsilyl chloride) (1.76 g, 11.7 mmol) were added at room temperature. The reaction mixture was stirred for 3 hours at room temperature, diluted with diethyl ether (200 mL) and washed with water (25 mL) and with saturated brine (25 mL), each three times. The thus obtained aqueous layer was extracted with 25 mL of diethyl ether three times. The ether layer was combined with the previously obtained organic layer, dried over magnesium sulfate, filtered and concentrated. The residue was purified by silica gel chromatography (ethyl acetate/hexane 1:9) to give Compound 9 (3.82 g, 99%) as a colorless oil.

$[\alpha]^{20}_D$  7.65 (c 1.00,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  0.02 (s, 3H), 0.03 (s, 3H), 0.83 (s, 9H), 1.18 (s, 9H), 2.80 (qt, 1H,  $J = 4.8, 6.8, 8.8$  Hz), 3.28 (s, 3H), 3.70 (dd, 1H,  $J = 6.8, 10.0$  Hz), 3.76 (dd, 1H,  $J = 8.8, 10.0$  Hz), 4.07 (d, 1H,  $J = 4.8$  Hz), 4.09 (dd, 1H,  $J = 7.4, 12.0$  Hz), 4.13 (dd, 1H,  $J = 4.8, 12.0$  Hz), 4.69 (d, 1H,  $J = 12.0$  Hz), 5.10 (s, 1H), 7.28 (m, 5H); HREIMS  $\text{C}_{25}\text{H}_{41}\text{O}_6\text{Si}$  ( $\text{M}^+ - \text{OCH}_3$ ) calcd. 465.2673, found 465.2666.

(9) Synthesis of 3S,4R,5R-4-(tert-butyldimethylsilyloxymethyl)-3-methoxymethyloxy-6-O-pivaloyl-hex-1-en-5-ol (Compound 10)

Compound 9 (3.98 g, 8.02 mmol) was dissolved in ethanol (40 mL), and  $\text{Pd}(\text{OH})_2$  (400 mg) was added, followed by stirring under hydrogen atmosphere for 12 hours at room



temperature for catalytic reduction. The reaction mixture was filtered through CELITE, and the filtrate was concentrated to give hemiacetal compound as a colorless oil which was subjected to the next reaction without further purification.

Methyltriphosponium bromide (10.9 g, 30.5 mL) was dissolved in THF (50 mL), cooled to 0°C under argon atmosphere, and 1.0 M LiHMDS (lithium bis(trimethylsilyl)amide) in THF (29 mL, 29.0 mmol) was added dropwise. The mixture was stirred at room temperature for 40 min. and then cooled again to 0°C when the color of the mixture was changed to yellow, followed by the addition of a THF solution of the hemiacetal (20 mL). The reaction mixture was stirred for 40 min. at 0°C, then MeOH (5 mL) was added. The mixture was diluted with diethyl ether (300 mL) and washed with saturated aqueous ammonium chloride solution (50 mL) and with saturated brine (50 mL), each three times. The organic layer was collected. The aqueous layer was extracted with 50 mL of diethyl ether three times. The ether layers were combined with the previously obtained ether layer, dried over magnesium sulfate, filtered and concentrated. The residue was purified by flash column chromatography (ethyl acetate/hexane 1:6) to give olefin Compound 10 (2.57 g, 79%) as a colorless oil.

$[\alpha]^{20}_D$  -5.63 (c 1.00, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 0.05 (s, 3H), 0.07 (s, 3H), 0.89 (s, 9H), 1.21 (s, 9H), 2.74 (ddt, 1H, J= 4.4, 7.2, 8.0 Hz), 3.40 (s, 3H), 3.54 (d, 1H,

J= 5.6 Hz), 3.82 (dd, 1H, J= 4.4, 10.4 Hz), 3.90 (dd, 1H, J= 4.8, 5.6, 7.2 Hz), 4.40 (t, 1H, J= 8.0 Hz), 4.58 (d, 1H, J= 6.4 Hz), 4.72 (d, 1H, J= 6.4 Hz), 5.30 (dd, 1H, J= 1.2, 16.8 Hz), 5.33 (dd, 1H, J= 1.2, 10.4 Hz), 5.70 (ddd, 1H, J= 8.0, 10.4, 16.8 Hz); HREIMS  $C_{19}H_{37}O_5Si$  ( $M^+-OCH_3$ ) calcd. 373.2411, found 373.2421.

(10) Synthesis of 3S,4R,5R-4-(tert-butyltrimethylsilyloxymethyl)-3-methoxymethyloxy-hex-1-en-5,6-diol (Compound 11)

Compound 10 (3.20 g, 7.92 mmol) was dissolved in dichloromethane (50 mL), and 1.0M DIBAL (diisobutyl aluminum hydride) in toluene (19.8 mL, 19.8 mmol) was added dropwise over 30 min. After 10 min., methanol (1 mL) and saturated aqueous ammonium chloride solution (1 mL) were added to the reaction mixture, which was then diluted with diethyl ether (250 mL) and filtered through CELITE. The filtrate was washed with a saturated aqueous ammonium chloride solution (50 mL) and with saturated brine (50 mL), each three times. The organic layer was collected. The aqueous layer was extracted with 25 mL of diethyl ether three times. The ether layers were combined with the previously obtained ether layer, dried over magnesium sulfate, filtered and concentrated. The residue was purified by flash column chromatography (ethyl acetate/hexane 2:3) to give diol Compound 11 (2.12 g, 84%) as a colorless oil.

$[\alpha]^{20}_D$  -6.89 (c 1.00,  $CHCl_3$ );  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  0.05 (s, 3H), 0.06 (s, 3H), 0.89 (s, 9H), 1.77 (ddt, 1H, J= 4.8,

6.5, 8.0 Hz), 2.67 (dd, 1H, J= 4.8, 8.4 Hz), 3.40(s, 3H),  
3.52 (d, 1H, J= 6.0 Hz), 3.70 (m, 1H), 3.83 (d, 1H, J= 4.8  
Hz), 4.14 (m, 1H), 4.30 (t, 1H, J= 8.0 Hz), 4.57 (d, 1H, J=  
6.8 Hz), 4.71 (d, 1H, J= 6.8 Hz), 5.29 (d, 1H, J= 16.8 Hz),  
5 5.32 (dd, 1H, J= 10.0 Hz), 5.69 (ddd, 1H, J= 8.0, 10.0,  
16.8 Hz); HREIMS  $C_{14}H_{29}O_4Si$  ( $M^+-OCH_3$ ) calcd. 289.1835, found  
289.1838.

(11) Synthesis of 3S,4R,5R-4-(tert-  
butyldimethylsilyloxymethyl)-3-methoxymethyloxy-hex-1-en-  
10 5,6-epoxide (Compound 12)

Compound 11 (3.20 g, 11.6 mmol) was dissolved in  
dichloromethane (40 mL), mixed with DMAP (4-  
(dimethylamino)pyridine) (2.84 g, 23.4 mmol), cooled to 0°C  
and vigorously stirred. To the mixture, 2-  
15 mesitylenesulfonyl chloride (3.80 g, 17.4 mmol) was slowly  
added, followed by stirring at that temperature for 4  
hours. The reaction mixture was diluted with diethyl ether  
(200 mL) and washed with water (25 mL) and with saturated  
brine (25 mL), each three times. The aqueous layer was  
20 extracted with 25 mL of diethyl ether three times. The  
ether layers were combined with the previously obtained  
ether layer, dried over magnesium sulfate, filtered and  
concentrated to give a sulfonate, which was used to the  
next step without further purification.

25 The sulfonate was dissolved in THF (50 mL), cooled to  
-78°C, 1.0 M LiHMDS in THF (2.5 mL, 2.5 mmol) was added  
dropwise. After stirring for 20 min. at that temperature,  
the mixture was warmed to 0°C and stirred for further 20

min. The reaction mixture was diluted with diethyl ether (200 mL), washed with a saturated aqueous ammonium chloride solution (25 mL) and with saturated brine (25 mL), each three times. The aqueous layer was extracted with 25 mL of diethyl ether three time. The ether layers were combined with the previously obtained diethyl ether layer, dried over magnesium sulfate, filtered and concentrated. The residue was purified by flash column chromatography (ethyl acetate/hexane 1:9) to give epoxy Compound 12 (3.01 g, 86%) as a colorless oil.

$[\alpha]_D^{20}$  -7.90 (c 1.00,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  0.02 (s, 3H), 0.04 (s, 3H), 0.87 (s, 9H), 1.26 (dddd, 1H,  $J$  = 3.4, 4.0, 5.2, 7.2 Hz), 2.62 (dd, 1H,  $J$  = 2.8, 5.2 Hz), 2.87 (t, 1H,  $J$  = 5.2 Hz), 3.04 (td, 1H,  $J$  = 2.8, 3.4, 5.2 Hz), 3.35 (s, 3H), 3.70 (dd, 1H,  $J$  = 4.0, 10.0 Hz), 3.82 (dd, 1H,  $J$  = 5.2, 10.0 Hz), 4.31 (t, 1H,  $J$  = 7.2 Hz), 4.51 (d, 1H,  $J$  = 6.4 Hz), 4.68 (d, 1H,  $J$  = 6.4 Hz), 5.24 (d, 1H,  $J$  = 10.2 Hz), 5.25 (d, 1H,  $J$  = 17.2 Hz), 5.70 (ddd, 1H,  $J$  = 7.2, 10.0, 17.2 Hz); HREIMS  $\text{C}_{14}\text{H}_{27}\text{O}_3\text{Si}$  ( $\text{M}^+ - \text{OCH}_3$ ) calcd. 271.1729, found 271.1732.

(12) Synthesis of 3S,4R,5R-4-(tert-butyldimethylsilyloxymethyl)-3-methoxymethyloxy-8-trimethylsilyl-oct-1-en-7-yn-5-ol (Compound 13)

Ethynyltrimethylsilane (3.88 g, 39.6 mmol) was dissolved in THF (100 mL), cooled to  $0^\circ\text{C}$ . 1.54M n-Butyl lithium-hexane (22.7 mL, 36.0 mmol) was slowly added. The mixture was stirred at that temperature for 15 min., then cooled to  $-78^\circ\text{C}$ . A solution of Compound 12 (3.01 g, 9.97

mmol) in THF (20 mL) and boron trifluoride diethyl ether complex (1.70 g, 12.0 mmol) were added. The mixture was warmed to room temperature, stirred for 40 min., diluted with diethyl ether (300 mL) and washed with a saturated aqueous ammonium chloride solution (50 mL) and with saturated brine (50 mL), each three times. The aqueous layer was extracted with diethyl ether (50 mL) three times. The ether layers were combined with the previously obtained ether layer, dried over magnesium sulfate, filtered and concentrated. The residue was purified by flash column chromatography (ethyl acetate/hexane 1:9) to give enyne Compound 13 (3.20 g, 80%) as a colorless oil.

$[\alpha]^{20}_D -7.14$  (c 1.00,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  0.04 (s, 3H), 0.05 (s, 3H), 0.11 (s, 9H), 0.87 (s, 9H), 1.93 (ddt, 1H,  $J = 4.0, 5.6, 8.0$  Hz), 2.46 (dd, 1H,  $J = 8.0, 16.8$  Hz), 2.63 (dd, 1H,  $J = 6.4, 16.8$  Hz), 3.38 (s, 3H), 3.60 (d, 1H,  $J = 5.6$  Hz), 3.81 (dd, 1H,  $J = 4.0, 10.0$  Hz), 3.92 (dd, 1H,  $J = 5.6, 10.8$  Hz), 4.21 (ddt, 1H,  $J = 5.6, 6.4, 8.0$  Hz), 4.44 (t, 1H,  $J = 8.0$  Hz), 4.58 (d, 1H,  $J = 6.4$  Hz), 4.70 (d, 1H,  $J = 6.4$  Hz), 5.28 (d, 1H,  $J = 17.2$  Hz), 5.29 (d, 1H,  $J = 10.8$  Hz), 5.71 (td, 1H,  $J = 8.0, 10.8, 17.2$  Hz).

(13) Synthesis of 3S,4R,5R-4-hydroxymethyl-3-methoxymethyloxy-oct-1-en-7-yn-5-ol (Compound 14)

Compound 13 (3.20 g, 8.0 mmol) was dissolved in THF (45 mL), cooled to  $0^\circ\text{C}$ , and 1.0M TBAF (tetrabutylammonium fluoride) in THF (17.6 mL, 17.6 mmol) was added. The reaction mixture was warmed to room temperature, stirred for further 1 hour, diluted with ethyl acetate (200 mL) and

washed with a saturated aqueous ammonium chloride solution (25 mL) and with saturated brine (25 mL), each three times. The aqueous layer was extracted with ethyl acetate (25 mL) three times. The ethylacetate layers were combined with the previously obtained organic layer, dried over magnesium sulfate, filtered and concentrated. The residue was purified by flash column chromatography (ethyl acetate/hexane 2:3) to give diol Compound 14 (1.69 g, 99%) as a colorless oil.

10  $[\alpha]_D^{20}$  -11.15 (c 1.00,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.86 (ddt, 1H, J= 3.2, 4.8, 7.2 Hz), 2.05 (t, 1H, J= 2.8 Hz), 2.50 (ddd, 1H, J= 2.8, 6.8, 16.8 Hz), 2.60 (bs, 1H), 2.63 (ddd, 1H, J= 2.8, 6.8, 16.8 Hz), 3.32 (d, 1H, J= 4.4 Hz), 3.34 (s, 3H), 3.83 (dd, 1H, J= 4.8, 11.2 Hz), 4.00 (dd, 1H, J= 4.8, 11.2 Hz), 4.30 (ddt, 1H, J= 3.2, 4.4, 6.8 Hz), 4.44 (tt, 1H, J= 1.2, 7.2 Hz), 4.61 (d, 1H, J= 6.8 Hz), 4.70 (d, 1H, J= 6.8 Hz), 5.33 (dt, 1H, J= 1.2, 10.4 Hz), 5.35 (d, 1H, J= 1.2, 17.2 Hz), 5.71 (ddd, 1H, J=7.2, 10.4, 17.2 Hz).  
15 (14) Synthesis of 3S,4R,5R-4-pivaloyloxymethyl-3-methoxymethyloxy-oct-1-en-7-yn-5-ol (Compound 15)  
20

Compound 14 (1.62 g, 7.57 mmol) was dissolved in pyridine (1.7 mL) and dichloromethane (6.8 mL) and cooled to 0°C. To the solution, trimethylacetyl chloride (1.08 g, 8.96 mmol) was added dropwise over 30 min., and stirred at the same temperature for 1 hour. The reaction mixture was warmed to room temperature, stirred for 4 hours, diluted with diethyl ether (100 mL) and washed with saturated aqueous ammonium chloride solution (20 mL) and with

2022011845001  
saturated brine (20 mL), each three times. The aqueous layer was extracted with diethyl ether (20 mL) three times. The ether layers were combined with the previously obtained ether layer, dried over magnesium sulfate, filtered and concentrated. The residue was purified by flash column chromatography (ethyl acetate/hexane 1:4) to give alcohol Compound 15 (1.92 g, 85%) as a colorless oil.

$[\alpha]_D^{20}$  -10.05 (c 1.00,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.22 (s, 9H), 2.03 (t, 1H,  $J$  = 8.0 Hz), 2.46 (ddt, 1H,  $J$  = 2.4, 4.8, 5.6 Hz), 2.46 (ddd, 1H,  $J$  = 2.4, 7.6, 16.8 Hz), 2.58 (ddd, 1H,  $J$  = 2.4, 6.4, 16.8 Hz), 3.10 (d, 1H,  $J$  = 2.4 Hz), 3.39 (s, 3H), 4.26 (ddt, 1H,  $J$  = 2.4, 6.4, 7.6 Hz), 4.29 (dd, 1H,  $J$  = 5.6, 11.6 Hz), 4.33 (dd, 1H,  $J$  = 5.6, 11.6 Hz), 4.40 (ddt, 1H,  $J$  = 1.2, 4.8, 6.8 Hz), 4.44 (t, 1H,  $J$  = 8.0 Hz), 4.57 (d, 1H,  $J$  = 7.2 Hz), 4.70 (d, 1H,  $J$  = 7.2 Hz), 5.31 (dt, 1H,  $J$  = 1.2, 17.6 Hz), 5.34 (d, 1H,  $J$  = 1.2, 10.4 Hz), 5.71 (ddd, 1H,  $J$  = 6.8, 10.4, 17.6 Hz).

(15) Synthesis of 3S,4R,5R-4-pivaloyloxymethyl-oct-1-en-7-yn-3,5-diol (Compound 16)

Compound 15 (2.10 g, 7.05 mmol) was dissolved in tert-butyl alcohol (60 mL). Pyridinium p-toluenesulfonate (17.6 g, 70.0 mmol) was added. The mixture was refluxed for 12 hours. The reaction mixture was cooled to room temperature and then concentrated. The residue was diluted with diethyl ether (200 mL) and washed with saturated sodium bicarbonate solution (20 mL) and with saturated brine (20 mL), each three times. The aqueous layer was extracted with diethyl ether (20 mL) three times. The

ether layers were combined with the previously obtained ether layer, dried over magnesium sulfate, filtered and concentrated. The residue was purified by flash column chromatography (ethyl acetate/hexane 1:3) to give diol

5 Compound 16 (1.33 g, 74%) as a colorless oil.

[ $\alpha$ ]<sub>D</sub><sup>20</sup> 1.75 (c 1.00, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.22 (s, 9H), 2.05 (t, 1H, J= 2.4 Hz), 2.06 (dddd, 1H, J= 2.4, 4.4, 5.6, 6.8 Hz), 2.43 (ddd, 1H, J= 2.4, 7.2, 16.8 Hz), 2.57 (ddd, 1H, J= 2.4, 7.2, 16.8 Hz), 2.73 (d, 1H, J= 4.4  
10 Hz), 3.07 (d, 1H, J= 2.4 Hz), 4.24 (tt, 1H, J= 2.4, 7.2 Hz), 4.31 (dd, 1H, J= 5.6, 11.6 Hz), 4.43 (dd, 1H, J= 6.8, 11.6 Hz), 4.45 (ddt, 1H, J= 1.2, 4.4, 5.6 Hz), 5.28 (dt, 1H, J= 1.2, 10.4 Hz), 5.28 (dt, 1H, J= 1.2, 10.4 Hz), 5.40 (d, 1H, J= 1.2, 16.8 Hz), 5.92 (ddd, 1H, J= 5.6, 10.4, 16.8  
15 Hz).

(16) Synthesis of 3S,4R,5R-4-pivaloyloxymethyl-3,5-di-(tert-butyldimethylsilyloxy)-oct-1-en-7-yne (Compound 17)

Compound 16 (1.31 g, 5.16 mmol) was dissolved in dichloromethane (25 mL), cooled to 0°C, and 2,6-lutidine  
20 (2.21 g, 20.6 mmol) and TBDMSOTf (tert-butyldimethylsilyltriflate) (4.09 g, 15.5 mmol) were added. The reaction mixture was stirred for 2 hours at the same temperature, diluted with diethyl ether (150 mL) and washed with a saturated aqueous sodium bicarbonate solution (20  
25 mL) and with saturated brine (20 mL), each three times. The aqueous layer was extracted with diethyl ether (20 mL) three times. The ether layers were combined with the previously obtained ether layer, dried over magnesium



sulfate, filtered and concentrated. The residue was purified by flash column chromatography (ethyl acetate/hexane 1:40) to give silyl ether Compound 17 (2.47 g, 99%) as a colorless oil.

5  $[\alpha]_D^{20}$  -0.37 (c 1.00,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  0.04 (s, 3H), 0.06 (s, 3H), 0.07 (s, 3H), 0.10 (s, 3H), 0.88 (s, 9H), 0.89 (s, 9H), 1.20 (s, 9H), 1.98 (t, 1H,  $J$  = 2.4 Hz), 2.19 (dq, 1H,  $J$  = 4.0, 6.0 Hz), 2.45 (ddd, 1H,  $J$  = 2.4, 4.8, 16.4 Hz), 2.54 (ddd, 1H,  $J$  = 2.4, 7.2, 16.4 Hz), 3.99 (dd, 1H,  $J$  = 6.0, 12.0 Hz), 4.16 (ddd, 1H,  $J$  = 4.0, 4.8, 7.2 Hz), 4.17 (dd, 1H,  $J$  = 6.0, 7.6 Hz), 4.25 (dd, 1H,  $J$  = 6.0, 12.0 Hz), 5.09 (d, 1H,  $J$  = 10.0 Hz), 5.16 (d, 1H,  $J$  = 17.2 Hz), 5.84 (ddd, 1H,  $J$  = 7.6, 10.0, 17.2 Hz); HREIMS  $\text{C}_{22}\text{H}_{41}\text{O}_4\text{Si}_2$  ( $\text{M}^+$ -t-Bu) calcd. 425.2543, found 425.2543

15 (17) Synthesis of 3S,4R,5R-4-hydroxymethyl-3,5-di-(tert-butyltrimethylsilyloxy)-oct-1-en-7-yne (Compound 18)

Compound 17 (2.47 g, 5.12 mmol) was dissolved in dichloromethane (20 mL) and cooled to  $-78^\circ\text{C}$ . 1.0M Diisobutyl aluminium hydride in toluene (7.7 mL, 7.70 mmol) was added dropwise over 30 min. The mixture was stirred at the same temperature for 10 min. Methanol (1 mL) and saturated aqueous ammonium chloride solution (1 mL) were added, and diluted with diethyl ether (200 mL). The mixture was filtered through CELITE. The filtrate was washed with saturated aqueous ammonium chloride solution (20 mL) and with saturated brine (20 mL), each three times. The aqueous layer was extracted with diethyl ether (20 mL) three times. The ether layers were combined with the

previously obtained ether layer, dried over magnesium sulfate, filtered and concentrated. The residue was purified by flash column chromatography (ethyl acetate/hexane 1:10) to give alcohol Compound 18 (1.96 g, 96%) as a colorless oil.

$[\alpha]_D^{20}$  0.11 (c 1.00,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  0.06 (s, 3H), 0.10 (s, 3H), 0.11 (s, 3H), 0.13 (s, 3H), 0.90 (s, 18H), 2.01 (t, 1H,  $J = 2.8$  Hz), 2.09 (ddt, 1H,  $J = 2.0, 4.8, 7.2$  Hz), 2.46 (ddd, 1H,  $J = 2.8, 4.8, 16.8$  Hz), 2.51 (ddd, 1H,  $J = 2.8, 6.8, 16.8$  Hz), 3.07 (dd, 1H,  $J = 4.8, 7.2$  Hz), 3.72 (ddd, 1H,  $J = 4.8, 7.2, 12.4$  Hz), 3.82 (dt, 1H,  $J = 7.2, 12.4$  Hz), 4.08 (dt, 1H,  $J = 4.8, 6.8$  Hz), 4.33 (dd, 1H,  $J = 6.0, 12.0$  Hz), 5.20 (dt, 1H,  $J = 2.0, 10.4$  Hz), 5.28 (dt, 1H,  $J = 2.0, 17.2$  Hz), 5.84 (ddd, 1H,  $J = 7.2, 10.4, 17.2$  Hz); HREIMS  $\text{C}_{21}\text{H}_{42}\text{O}_3\text{Si}_2$  ( $M^+$ ) calcd. 398.2672, found 398.2669.

(18) Synthesis of 3S,4R,5R-4-(tert-butyldimethylsilyloxymethyl)-3,5-di-(tert-butyldimethylsilyloxy)-oct-1-en-7-yne (Compound 19)

Compound 18 (40.0 mg, 101  $\mu\text{mol}$ ) was dissolved in DMF (2.0 mL), and imidazole (13.6 mg, 200  $\mu\text{mol}$ ) and TBDMSCl (tert-butyldimethylsilyl chloride) (22.6 mg, 150  $\mu\text{L}$ ) were added at room temperature. The mixture was stirred for 3 hours. The reaction mixture was diluted with diethyl ether (20 mL) and washed with water (5 mL) and with saturated brine (5 mL), each three times. The aqueous layer was extracted with diethyl ether (5 mL) three times. The ether layers were combined with the previously obtained ether

layer, dried over magnesium sulfate, filtered and concentrated. The residue was purified by flash column chromatography (ethyl acetate/hexane 1:50) to give silyl ether Compound 19 (46.0 mg, 88%) as a colorless oil.

5  $[\alpha]_D^{20}$  0.59 (c 1.00,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  0.03 (s, 3H), 0.04 (s, 3H), 0.06 (s, 3H), 0.10 (s, 3H), 0.89 (s, 9H), 0.90 (s, 18H), 1.93 (t, 1H,  $J = 2.8$  Hz), 2.00 (ddt, 1H,  $J = 5.2, 5.6, 6.4$  Hz), 2.44 (ddd, 1H,  $J = 2.8, 5.6, 16.8$  Hz), 2.57 (ddd, 1H,  $J = 2.8, 5.6, 16.8$  Hz), 3.56 (dd, 1H,  $J = 6.0, 10.0$  Hz), 3.81 (dd, 1H,  $J = 6.0, 10.0$  Hz), 4.09 (dt, 1H,  $J = 4.8, 5.6$  Hz), 4.30 (ddt, 1H,  $J = 1.2, 5.2, 6.8$  Hz), 5.03 (dt, 1H,  $J = 1.2, 9.6$  Hz), 5.11 (dt, 1H,  $J = 1.2, 17.2$  Hz), 5.92 (ddd, 1H,  $J = 6.8, 9.6, 17.2$  Hz).

10 (19) Synthesis of (3S,4R,5R-4-cyanomethyl-3,5-di-(tert-butyl)dimethylsilyloxy)-oct-1-en-7-yne (Compound 20)

15 Compound 18 (314 mg, 789  $\mu\text{mol}$ ) was dissolved in dichloromethane, and DMAP (dimethylaminopyridine) (372 mg, 3.04 mmol) was added, followed by cooling to  $0^\circ\text{C}$ . 2-Mesitylenesulfonyl chloride (582 mg, 2.66 mmol) was added  
20 with vigorous stirring. The mixture was stirred for 12 hours at the same temperature. The reaction mixture was diluted with diethyl ether (100 mL) and washed with water (15 mL) and with saturated brine (15 mL), each three times. The aqueous layer was extracted with diethyl ether (15 mL)  
25 three times. The ether layers were combined with the previously obtained ether layer, dried over magnesium sulfate, filtered and concentrated. The thus obtained residue was subjected to the next step without further

purification.

The sulfon ester was dissolved in DMSO (dimethylsulfoxide) (5.0 mL), and sodium cyanide (78.0 mg, 1.59 mmol) was added, followed by stirring at 70°C for 2 hours. The reaction mixture was cooled to room temperature, diluted with diethyl ether (100 mL) and washed with water (15 mL) and with saturated brine (15 mL), each three times. The aqueous layer was extracted with diethyl ether (15 mL) three times. The ether layers were combined with the previously obtained ether layer, dried over magnesium sulfate, filtered and concentrated. The residue was purified by flash column chromatography (ethyl acetate/hexane 1:20) to give nitrile Compound 20 (263 mg, 82%) as a colorless oil.

[ $\alpha$ ]<sub>D</sub><sup>20</sup> -0.44 (c 1.00, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  0.06 (s, 3H), 0.09 (s, 3H), 0.12 (s, 3H), 0.13 (s, 3H), 0.90 (s, 9H), 0.91 (s, 9H), 2.03 (t, 1H, J= 2.8 Hz), 2.29 (tt, 1H, J= 6.0, 6.8 Hz), 2.36 (dd, 1H, J= 6.0, 16.4 Hz), 2.46 (ddd, 1H, J= 2.8, 4.4, 16.8 Hz), 2.48 (dd, 1H, J= 6.0, 16.4 Hz), 2.52 (ddd, 1H, J= 2.8, 6.8, 16.8 Hz), 4.04 (dt, 1H, J = 4.4, 6.8 Hz), 4.20 (ddt, 1H, J= 1.2, 6.4, 6.8 Hz), 5.24 (dt, 1H, J= 1.2, 10.4 Hz), 5.31 (dt, 1H, J = 1.2, 16.8 Hz), 5.82 (ddd, 1H, J= 6.4, 10.4, 16.8 Hz).

(20) Synthesis of 3S,4R,5R-3,5-di-(tert-butylldimethylsilyloxy)-4-(2'-ethanal)-oct-1-en-7-yne (Compound 21)

Compound 20 (184 mg, 452  $\mu$ mol) was dissolved in dichloromethane (2.0 mL), cooled to -78°C, and 1.0M

diisobutyl aluminium hydride in toluene (530  $\mu$ L, 530  $\mu$ mol) was added over 10 min., followed by stirring for 1 hour at that temperature. Methanol (1 mL) and a saturated aqueous ammonium chloride solution (1 mL) were added to the  
5 reaction mixture, which was then diluted with diethyl ether (100 mL). The mixture was filtered through CELITE. The filtrate was washed with saturated aqueous ammonium chloride solution (10 mL) and saturated brine (10 mL), each three times. The aqueous layer was extracted with diethyl  
10 ether (10 mL) three times. The ether layers were combined with the previously obtained ether layer, dried over magnesium sulfate, filtered and concentrated. The residue was purified by flash column chromatography (ethyl acetate/hexane 1:20) to give aldehyde Compound 21 (157 mg,  
15 85%) as a colorless oil.

$[\alpha]_D^{20}$  0.77 (c 1.00,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  0.04 (s, 3H), 0.06 (s, 3H), 0.08 (s, 3H), 0.09 (s, 3H), 0.88 (s, 9H), 0.89 (s, 9H), 2.04 (t, 1H,  $J$  = 2.8 Hz), 2.37 (ddd, 1H,  $J$  = 2.8, 6.0, 16.8 Hz), 2.40 (ddd, 1H,  $J$  = 2.8, 6.4, 17.2  
20 Hz), 2.42 (ddd, 1H,  $J$  = 1.6, 7.2, 17.2 Hz), 2.44 (ddd, 1H,  $J$  = 2.8, 6.0, 16.8 Hz), 2.68 (ddd, 1H,  $J$  = 5.2, 6.4 Hz), 3.84 (dt, 1H,  $J$  = 5.2, 6.0 Hz), 4.23 (ddt, 1H,  $J$  = 1.6, 6.4, 6.8 Hz), 5.18 (dt, 1H,  $J$  = 1.6, 10.0 Hz), 5.21 (dt, 1H,  $J$  = 1.6, 16.0 Hz), 5.73 (ddd, 1H,  $J$  = 6.8, 10.0, 16.8 Hz); HREIMS  
25  $\text{C}_{22}\text{H}_{42}\text{O}_3\text{Si}_2$  ( $\text{M}^+$ ) calcd. 410.2673, found 410.2667.

(21) Synthesis of 3S,4R,5R-4-(2'-hydroxyethyl)-3,5-di-(tert-butyldimethylsilyloxy)-oct-1-en-7-yne (Compound 22)

Compound 21 (157 mg, 383  $\mu$ mol) was dissolved in

methanol (2.0 mL), cooled to 0°C, and sodium borohydride (28.0 mg, 741 µmol) was added, followed by stirring at the same temperature for 30 min. The reaction mixture was warmed to room temperature, diluted with diethyl ether (100 mL) and washed with water (10 mL) and with saturated brine (10 mL), each three times. The aqueous layer was extracted with diethyl ether (10 mL) three times. The ether layers were combined with the previously obtained ether layer, dried over magnesium sulfate, filtered and concentrated.

The residue was purified by flash column chromatography (ethyl acetate/hexane 1:9) to give alcohol Compound 22 (129 mg, 82%) as a colorless oil.

$[\alpha]_D^{20}$  1.40 (c 1.00, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 0.08 (s, 6H), 0.10 (s, 6H), 0.90 (s, 9H), 0.91 (s, 9H), 1.67 (m, 2H), 2.03 (t, 1H, J = 2.8 Hz), 2.29 (tt, 1H, J = 6.0, 6.8 Hz), 2.36 (dd, 1H, J = 6.0, 16.4 Hz), 2.01 (t, 1H, J = 2.4 Hz), 2.04 (ddt, 1H, J = 4.8, 5.6, 7.2 Hz), 2.41 (ddd, 1H, J = 2.4, 5.6, 17.2 Hz), 2.45 (ddd, 1H, J = 2.4, 5.6, 17.2 Hz), 2.93 (bs, 1H), 3.65 (m, 2H), 3.85 (q, 1H, J = 5.6 Hz), 4.27 (ddt, 1H, J = 1.2, 4.8, 6.8 Hz), 5.18 (dt, 1H, J = 1.2, 10.8 Hz), 5.21 (dt, 1H, J = 1.2, 17.6 Hz), 5.86 (ddd, 1H, J = 6.8, 10.8, 17.6 Hz).

(22) Synthesis of 3S,4R,5R-4-(2'-tert-butyl dimethylsilyloxyethyl)-3,5-di-(tert-butyl dimethylsilyloxy)-oct-1-en-7-yne (Compound 23)

From Compound 22 (60.0 mg, 146 µmol), silyl ether Compound 23 (59.0 mg, 78%) was obtained according to the same procedure of Compound 19.

[ $\alpha$ ]<sub>D</sub><sup>20</sup> 0.90 (c 1.00, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  0.03 (s, 3H), 0.04 (s, 6H), 0.05 (s, 3H), 0.07 (s, 3H), 0.90 (s, 3H), 0.89 (s, 27H), 1.55 (m, 2H), 1.84 (ddt, 1H, J= 3.6, 5.2, 6.4 Hz), 1.95 (t, 1H, J= 2.8 Hz), 2.36 (ddd, 1H, J= 2.8, 6.4, 16.8 Hz), 2.41 (ddd, 1H, J= 2.8, 6.4, 16.8 Hz), 3.57 (dt, 1H, J= 6.4, 8.4 Hz), 3.69 (dd, 1H, J= 6.4, 10.0 Hz), 4.01 (dt, 1H, J= 3.6, 6.4 Hz), 4.13 (t, 1H, J = 6.4 Hz), 5.11 (d, 1H, J= 10.4 Hz), 5.17 (d, 1H, J= 17.2 Hz), 5.82 (ddd, 1H, J= 6.4, 10.4, 17.2 Hz).

(23) Synthesis of 3S,4R,5R-3,5-di-(tert-butyldimethylsilyloxy)-4-ethyl-oct-1-en-7-yne (Compound 24)

Compound 22 (100 mg, 243  $\mu$ mol) was dissolved in dichloromethane (2.0 mL), and DMAP (74.0 mg, 606  $\mu$ mol) was added. The mixture was cooled to 0°C. 2-Mesitylene sulfonyl chloride (106 mg, 485  $\mu$ mol) was slowly added with vigorous stirring, and then stirred for 12 hours at the same temperature. The reaction mixture was diluted with diethyl ether (100 mL) and washed with water (10 mL) and with saturated brine (10 mL), each three times. The aqueous layer was extracted with diethyl ether (10 mL) three times. The ether layers were combined with the previously obtained ether layer, dried over magnesium sulfate, filtered and concentrated. The thus obtained sulfon ester was subjected to the next step without further purification.

The sulfon ester was dissolved in diethyl ether (2.0 mL), cooled to 0°C. LAH (lithium aluminium hydride) (46.0 mg, 1.20  $\mu$ mol) was slowly added. The mixture was stirred

at the same temperature for 1 hour and at room temperature for further 3 hours. The reaction mixture was cooled to 0°C, and ethyl acetate (1 mL) and saturated aqueous ammonium chloride solution (1 mL) were added, followed by

5 diluting with diethyl ether (100 mL). The mixture was filtered through CELITE. The filtrate was washed with saturated aqueous ammonium chloride solution (10 mL) and saturated brine (10 mL), each three times. The aqueous layer was extracted with diethyl ether (10 mL) three times.

10 The ether layers were combined with the previously obtained ether layer, dried over magnesium sulfate, filtered and concentrated. The residue was purified by flash column chromatography (ethyl acetate/hexane 1:100) to give enyne Compound 24 (77.0 mg, 80%) as a colorless oil.

15  $[\alpha]^{20}_D$  1.95 (c 1.00, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 0.03 (s, 3H), 0.05 (s, 3H), 0.07 (s, 3H), 0.09 (s, 3H), 0.89 (s, 18H), 0.94 (t, 3H, J= 7.6 Hz), 1.36 (m, 2H), 1.68 (ddt, 1H, J= 3.6, 5.2, 6.0 Hz), 1.95 (t, 1H, J= 2.8 Hz), 2.39 (ddd, 1H, J= 2.8, 6.4, 16.8 Hz), 2.42 (ddd, 1H, J= 2.8, 6.4, 16.8 Hz), 4.01 (dt, 1H, J= 3.6, 6.4 Hz), 4.14 (ddt, 1H, J= 1.2, 5.2, 7.2 Hz), 5.08 (dt, 1H, J= 1.2, 10.4 Hz), 5.14 (dt, 1H, J= 1.2, 16.8 Hz), 5.86 (ddd, 1H, J= 7.2, 10.4, 16.8 Hz).

20 (24) Synthesis of 3S,4R,5R-3,5-di-(tert-butyltrimethylsilyloxy)-4-(2'-cyanoethyl)-oct-1-en-7-yne  
25 (Compound 25)

From Compound 22 (129 mg, 313 μmol), nitrile Compound 25 (80.0 mg, 61%) was obtained as a colorless oil according to the same procedure of Compound 20.



202207181500T  
[ $\alpha$ ]<sub>D</sub><sup>20</sup> 1.40 (c 1.00, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  0.05 (s, 3H), 0.08 (s, 6H), 0.10 (s, 3H), 0.90 (s, 18H), 1.73 (m, 2H), 1.96 (q, 1H, J= 6.4 Hz), 2.03 (t, 1H, J= 2.4 Hz), 2.38 (ddd, 1H, J= 2.4, 6.0, 16.8 Hz), 2.43 (ddd, 1H, J= 2.4, 4.8, 16.8 Hz), 2.50 (dt, 2H, J= 4.0, 8.4 Hz), 3.88 (dt, 1H, J= 4.8, 6.0 Hz), 4.18 (tt, 1H, J= 1.2, 6.4 Hz), 5.19 (dt, 1H, J = 1.2, 10.4 Hz), 5.25 (dt, 1H, J= 1.2, 17.2 Hz), 5.80 (ddd, 1H, J= 6.4, 10.4, 17.2 Hz) ; HREIMS C<sub>23</sub>H<sub>43</sub>O<sub>2</sub>NSi<sub>2</sub> (M<sup>+</sup>) calcd. 421.2814, found 421.2812.

10 (25) Synthesis of 3S,4R,5R-3,5-di-(tert-butyltrimethylsilyloxy)-4-(3'-propanal)-oct-1-en-7-yne (Compound 26)

From the nitrile Compound 25 (80.0 mg, 190  $\mu$ mol), aldehyde Compound 26 (71.0 mg, 89%) was obtained as a colorless oil according to the same procedure of Compound 21.

15 [  $\alpha$  ]<sub>D</sub><sup>20</sup> 0.37 (c 1.00, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  0.04 (s, 3H), 0.06 (s, 3H), 0.07 (s, 3H), 0.10 (s, 3H), 0.89 (s, 18H), 1.69 (m, 2H), 1.87 (ddt, 1H, J= 5.2, 5.6, 6.0 Hz), 1.87 (ddt, 1H, J= 5.2, 5.6, 6.0 Hz), 1.99 (t, 1H, J= 2.8 Hz), 2.40 (ddd, 1H, J= 2.8, 6.0, 17.2 Hz), 2.43 (ddd, 1H, J= 2.8, 6.0, 17.2 Hz), 2.57 (ddd, 1H, J= 2.0, 2.8, 6.0 Hz), 2.59 (ddd, 1H, J= 2.0, 3.2, 6.0 Hz), 3.94 (dt, 1H, J = 4.4, 6.0 Hz), 4.17 (ddt, 1H, J= 1.6, 5.6, 6.8 Hz), 5.14 (dt, 1H, J= 1.6, 10.4 Hz), 5.25 (dt, 1H, J= 1.6, 17.2 Hz), 5.80 (ddd, 1H, J= 6.8, 10.4, 17.2 Hz).

25 (26) Synthesis of 3S,4R,5R-4-(3'-hydroxypropyl)-3,5-di-(tert-butyltrimethylsilyloxy)-oct-1-en-7-yne (Compound 27)

From the aldehyde Compound 26 (71.0 mg, 167  $\mu$ mol), alcohol Compound 27 (70.0 mg, 98%) was obtained as a colorless oil according to the same procedure of Compound 22.

5  $[\alpha]^{20}_D$  1.18 (c 1.00,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  0.04 (s, 3H), 0.06 (s, 3H), 0.07 (s, 3H), 0.10 (s, 3H), 0.89 (s, 18H), 1.39 (m, 2H), 1.57 (bs, 1H), 1.66 (q, 1H,  $J$  = 6.4 Hz), 1.85 (ddt, 1H,  $J$  = 4.8, 5.2, 5.6 Hz), 1.98 (t, 1H,  $J$  = 2.8 Hz), 2.39 (ddd, 1H,  $J$  = 2.8, 6.4, 16.4 Hz), 2.43 (ddd, 1H,  $J$  = 2.8, 6.4, 16.4 Hz), 3.61 (t, 2H,  $J$  = 6.4 Hz), 3.97 (dt, 1H,  $J$  = 4.8, 6.4 Hz), 4.16 (ddt, 1H,  $J$  = 1.2, 5.6, 7.2 Hz), 5.12 (dt, 1H,  $J$  = 1.2, 9.6 Hz), 5.17 (dt, 1H,  $J$  = 1.6, 16.8 Hz), 5.84 (ddd, 1H,  $J$  = 7.2, 9.6, 16.8 Hz); HREIMS  $\text{C}_{23}\text{H}_{46}\text{O}_3\text{Si}_2$  ( $M^+$ ) calcd. 426.2985, found 426.2977.

15 (27) Synthesis of 3S,4R,5R-4-(3'-tert-butyl dimethylsilyloxypropyl)-3,5-di-(tert-butyl dimethylsilyloxy)-oct-1-en-7-yne (Compound 28)

From the alcohol Compound 27 (60.0 mg, 141  $\mu$ mol), silyl ether Compound 28 (63.0 mg, 83%) was obtained as a colorless oil according to the same procedure of Compound 23.

20  $[\alpha]^{20}_D$  1.00 (c 1.00,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  0.03 (s, 3H), 0.04 (s, 6H), 0.05 (s, 3H), 0.07 (s, 3H), 0.09 (s, 3H), 0.89 (s, 27H), 1.32 (m, 2H), 1.56 (m, 2H), 1.75 (ddt, 1H,  $J$  = 4.0, 6.4, 6.8 Hz), 1.95 (t, 1H,  $J$  = 2.8 Hz), 2.38 (ddd, 1H,  $J$  = 2.8, 6.4, 16.8 Hz), 2.42 (ddd, 1H,  $J$  = 2.8, 6.4, 16.8 Hz), 3.56 (t, 2H,  $J$  = 6.8 Hz), 4.03 (dt, 1H,  $J$  = 3.6, 6.0 Hz), 4.12 (dd, 1H,  $J$  = 6.4, 7.6 Hz), 5.08 (d, 1H,

J= 10.0 Hz), 5.14 (d, 1H, J= 17.2 Hz), 5.84 (ddd, 1H, J= 7.6, 10.0, 17.2 Hz).

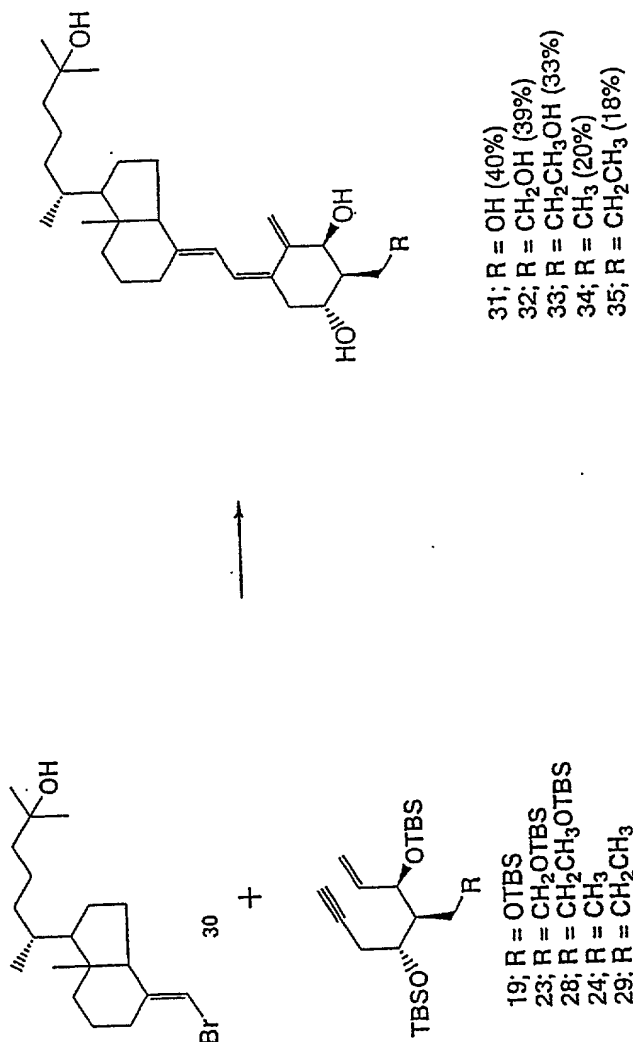
(28) Synthesis of 3S,4R,5R-3,5-di-(tert-butyltrimethylsilyloxy)-4-propyl-oct-1-en-7-yne (Compound 29)

From the alcohol Compound 27 (70.0 mg, 164  $\mu$ mol), enyne Compound 29 (54.0 mg, 80%) was obtained as a colorless oil according to the same procedure of Compound 24.

[ $\alpha$ ]<sub>D</sub><sup>20</sup> 2.24 (c 1.00, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  0.02 (s, 3H), 0.05 (s, 6H), 0.06 (s, 3H), 0.09 (s, 3H), 0.87 (t, 3H, J= 7.2 Hz), 0.89 (s, 18H), 1.27 (m, 2H), 1.36 (m, 2H), 1.76 (ddt, 1H, J= 4.0, 5.2, 6.0 Hz), 1.95 (t, 1H, J= 2.8 Hz), 2.39 (ddd, 1H, J= 2.8, 6.4, 17.6 Hz), 2.41 (ddd, 1H, J= 2.8, 6.4, 17.6 Hz), 3.99 (dt, 1H, J= 4.0, 6.4 Hz), 4.12 (ddt, 1H, J= 1.2, 6.0, 8.0 Hz), 5.07 (dt, 1H, J= 1.2, 10.4 Hz), 5.13 (dt, 1H, J= 1.2, 17.2 Hz), 5.85 (ddd, 1H, J= 8.0, 10.4, 17.2 Hz).

(Example 2) Synthesis of vitamin D derivatives having a substituent at the 2 $\alpha$ -position.

Example 2 was done by the following reaction scheme:



(1) Synthesis of (5Z,7E)-(1S,2S,3R,20R)-9,10-seco-5,7,10(19)-cholestatriene-2-hydroxymethyl-1,3,25-triol (Compound 31)

The silyl ether Compound 19 (20.0 mg, 39.1  $\mu$ mol) and a vinyl bromide 30 (20.0 mg, 58.0  $\mu$ mol), which corresponds to a compound of Formula (I) in which R<sup>1</sup> is a 4-hydroxy-4-methylpentyl group, were dissolved in triethylamine/toluene (3:1, 2.0 mL). Tris(dibenzylideneacetone)-dipalladium(0)-

chloroform addition product (4.0 mg, 3.86  $\mu\text{mol}$ ) and triphenylphosphine (10.0 mg, 38.1  $\mu\text{mol}$ ) were added. The mixture was stirred at room temperature for 15 min. and then refluxed for 2 hours. The reaction mixture was  
5 filtered through silica gel; the filtrate was concentrated and roughly purified by thin layer chromatography (ethyl acetate/hexane 1:4) to give a protected derivative as a colorless solid. The crude protected body was subjected to the next step without further purification.

10 The protected derivative was dissolved in methanol (2.0 mL), cooled to 0°C and (+)-10-camphorsulfonic acid (10.0 mg, 43  $\mu\text{mol}$ ) was added. The mixture was stirred at the same temperature for 1 hour and then returned to room temperature, followed by stirring for further 12 hours.

15 The reaction mixture was diluted with ethyl acetate (10 mL) and washed with saturated aqueous sodium bicarbonate solution (1.0 mL) and saturated brine (1.0 mL), each three times. The aqueous layer was extracted with ethyl acetate (2 mL) three times. The ethylacetate layers were combined  
20 with the previously obtained organic layer, dried over magnesium sulfate, filtered and concentrated. The residue was purified by thin layer chromatography (ethyl acetate/hexane 1:4) to give Compound 31 (7.0 mg, 40%) as a colorless solid.

25  $[\alpha]_D^{20}$ , 12.95 (c 0.0085,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  0.53 (s, 3H), 0.94 (d, 3H,  $J = 6.8$  Hz), 2.31 (m, 2H), 2.67 (dd, 1H,  $J = 4.4, 12.8$  Hz), 2.73 (bs, 1H), 2.84 (m, 1H), 4.01 (m, 2H), 4.24 (m, 1H), 4.47 (bs, 1H), 5.02 (d, 1H,  $J =$

2.0 Hz), 5.30 (d, 1H, J= 2.0 Hz), 5.98 (d, 1H, J= 10.8 Hz),  
6.45 (d, 1H, J= 10.8 Hz) ; HREIMS  $C_{28}H_{44}O_3$  ( $M^+ - H_2O$ ) calcd.  
428.3290, found 428.3287.

(2) Synthesis of (5Z,7E)-(1S,2S,3R,20R)-9,10-seco-

5,7,10(19)-cholestatriene-2-(2'-hydroxyethyl)-1,3,25-triol  
(Compound 32)

From the silyl ether Compound 23 (25.0 mg, 47.5  
 $\mu$ mol), Compound 32 (8.5 mg, 39%) was obtained as a  
colorless solid according to the same procedure of Compound  
31.

$[\alpha]_D^{20}$ , 13.85 (c 0.00722,  $CHCl_3$ );  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$   
0.53 (s, 3H), 0.94 (d, 3H, J= 6.4 Hz), 2.26 (dd, 1H, J=  
8.0, 12.8 Hz), 2.53 (bs, 1H), 2.66 (dd, 1H, J= 4.0, 13.2  
Hz), 2.83 (m, 1H), 3.79 (m, 2H), 3.94 (m, 1H), 4.37 (d, 1H,  
J= 2.0 Hz), 5.02 (d, 1H, J= 1.2 Hz), 5.30 (bs, 1H), 6.01  
(d, 1H, J= 10.8 Hz), 6.40 (d, 1H, J= 10.8 Hz) ; HREIMS  
 $C_{29}H_{48}O_4$  ( $M^+$ ) calcd. 460.3553, found 460.3557.

(3) Synthesis of (5Z,7E)-(1S,2S,3R,20R)-9,10-seco-

5,7,10(19)-cholestatriene-2-(3'-hydroxypropyl)-1,3,25-  
triol(Compound 33)

From the silyl ether Compound 28 (25.0 mg, 46.3  
 $\mu$ mol), Compound 33 (7.2 mg, 33%) was obtained as a  
colorless solid according to the same procedure of Compound  
31.

$[\alpha]_D^{20}$ , 161.29 (c 0.00186,  $CHCl_3$ );  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$   
0.53 (s, 3H), 0.94 (d, 3H, J= 6.4 Hz), 2.25 (dd, 1H, J=  
8.4, 13.2 Hz), 2.66 (dd, 1H, J= 4.0, 13.2 Hz), 2.83 (m,  
1H), 3.70 (t, 2H, J= 5.6 Hz), 3.90 (dt, 1H, J= 4.0, 8.0

Hz), 4.38 (d, 1H, J= 3.6 Hz), 5.00 (d, 1H, J= 1.6 Hz), 5.28 (d, 1H, J= 1.6 Hz), 6.00 (d, 1H, J= 11.2 Hz), 6.40 (d, 1H, J= 11.2 Hz).

(4) Synthesis of (5Z,7E)-(1S,2S,3R,20R)-9,10-seco-  
5 5,7,10(19)-cholestatriene-2-ethyl-1,3,25-triol (Compound  
34)

From the enyne Compound 24 (30.0 mg, 75.8  $\mu$ mol),  
Compound 34 (6.8 mg, 20%) was obtained as a colorless solid  
according to the same procedure of Compound 31.

10  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  0.53 (s, 3H), 0.94 (d, 3H, J= 6.0  
Hz), 0.95 (t, 3H, J= 7.2 Hz), 2.24 (dd, 1H, J= 8.8, 12.8  
Hz), 2.66 (dd, 1H, J= 4.0, 13.2 Hz), 2.83 (m, 1H), 3.89 (m,  
1H), 4.37 (bs, 1H), 4.99 (d, 1H, J= 1.6 Hz), 5.27 (bs, 1H),  
6.00 (d, 1H, J= 11.2 Hz), 6.40 (d, 1H, J= 11.2 Hz); HREIMS  
15  $\text{C}_{29}\text{H}_{48}\text{O}_3$  ( $\text{M}^+$ ) calcd. 444.3603, found 460.3604.

(5) Synthesis of (5Z,7E)-(1S,2S,3R,20R)-9,10-seco-  
5,7,10(19)-cholestatriene-2-propyl-1,3,25-triol (Compound  
35)

From the enyne Compound 29 (30.0 mg, 73.2  $\mu$ mol),  
20 Compound 35 (6.2 mg, 18%) was obtained as a colorless solid  
according to the same procedure of Compound 31.

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  0.53 (s, 3H), 0.94 (d, 3H, J= 6.4  
Hz), 1.01 (t, 3H, J= 6.8 Hz), 2.24 (dd, 1H, J= 8.8, 12.4  
Hz), 2.66 (dd, 1H, J= 4.0, 13.2 Hz), 2.83 (m, 1H), 3.89 (m,  
25 1H), 4.39 (bs, 1H), 4.99 (d, 1H, J= 1.6 Hz), 5.27 (bs, 1H),  
6.00 (d, 1H, J= 11.2 Hz), 6.40 (d, 1H, J= 11.2 Hz). ;  
HREIMS  $\text{C}_{30}\text{H}_{50}\text{O}_3$  ( $\text{M}^+$ ) calcd. 458.3760, found 458.3755.

(Test example 1) Assay for binding to vitamin D receptor (VDR)

Bovine thymus  $1\alpha,25$ -dihydroxyvitamin  $D_3$  receptor was purchased from YAMASA SHOYU KABUSHIKI KAISHA (lot.110431) and 1 ampule (approximately 25 mg) of the receptor was dissolved in 55 mL of 0.05 M phosphate 0.5M potassium buffer (pH 7.4) just before use. Ethanol solutions (50  $\mu$ l) of  $1\alpha,25$ -dihydroxyvitamin  $D_3$  and Compounds 31-35 of the present invention at various concentrations were prepared, mixed with aliquots (500  $\mu$ l, 0.23 mg protein) of the receptor solution and pre-incubated at room temperature for 1 hour. To the resultant mixtures, [ $^3$ H]- $1\alpha,25$ -dihydroxyvitamin  $D_3$  was added at the final concentration of 0.1 nM, followed by incubation overnight at 4°C.

The reaction mixtures were treated with dextran coated charcoal for 30 minutes at 4°C to separate the bound and free forms of [ $^3$ H]- $1\alpha,25$ -dihydroxyvitamin  $D_3$ , and centrifuged at 3000 rpm for ten minutes. Each of the resultant supernatants (500  $\mu$ l) was mixed with ACS-II (9.5 ml) (AMERSHAM, England) for radioactivity measurement.

The binding properties of Compounds 31-35 of the present invention were expressed in relative values with that of  $1\alpha,25$ -dihydroxyvitamin  $D_3$  taken as 100. The results are shown in Table 1.



Table 1

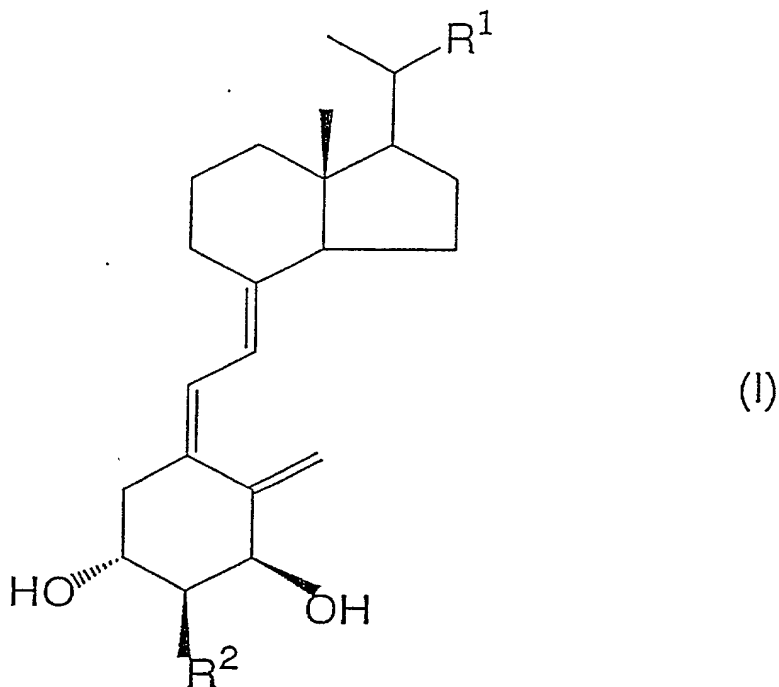
Compound	Binding property
31	20
32	70
33	300
34	40
35	20

#### INDUSTRIAL APPLICABILITY

5 Vitamin D<sub>3</sub> derivatives represented by Formula (I), (II) and (III) of the present invention are novel compounds and may be useful as pharmaceutical agents. The compounds of the present invention may be useful as reagents for studying metabolism of active vitamin D<sub>3</sub>, that is, 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub>.

# CLAIMS

1. A vitamin D derivative represented by Formula (I):

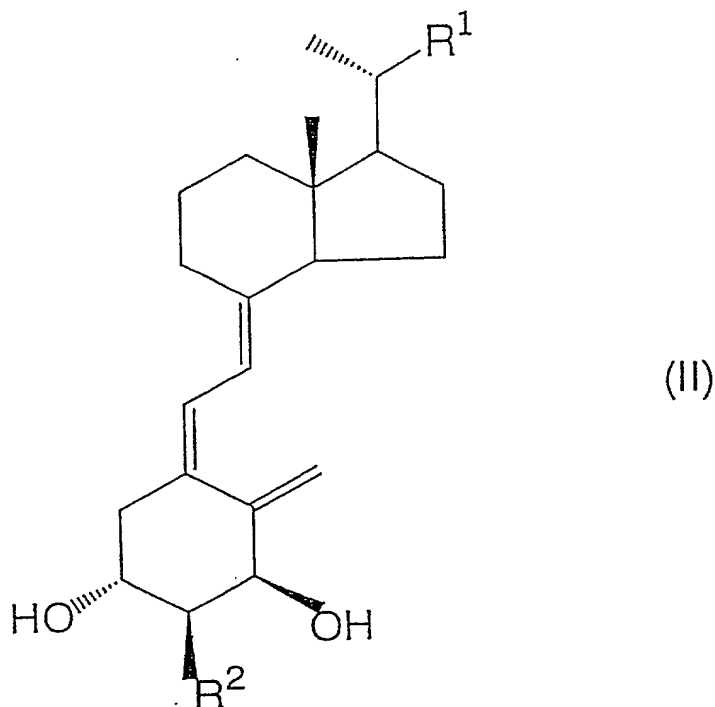


wherein

$R^1$  represents a saturated aliphatic  $C_{1-15}$  hydrocarbon group optionally substituted with 1 to 3 hydroxy or protected hydroxy groups; and

$R^2$  represents a saturated aliphatic  $C_{1-10}$  hydrocarbon group optionally substituted with one or more substituents, which may be the same or different and which are selected from the group consisting of a hydroxy group, a halogen atom, a cyano group, a lower alkoxy group, an amino group and an acylamino group, provided that when  $R^2$  represents a saturated aliphatic  $C_1$  hydrocarbon group,  $R^2$  is substituted with at least one substituent.

2. The vitamin D derivative of claim 1 which is represented by Formula (II):

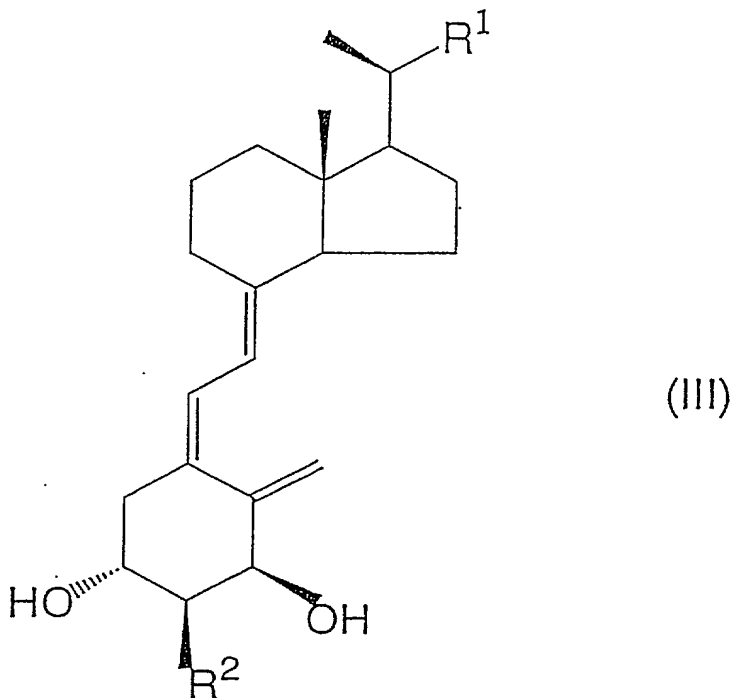


wherein

R<sup>1</sup> represents a saturated aliphatic C<sub>1-15</sub> hydrocarbon group optionally substituted with 1 to 3 hydroxy or protected hydroxy groups; and

R<sup>2</sup> represents a saturated aliphatic C<sub>1-10</sub> hydrocarbon group optionally substituted with one or more substituents, which may be the same or different and which are selected from the group consisting of a hydroxy group, a halogen atom, a cyano group, a lower alkoxy group, an amino group and an acylamino group, provided that when R<sup>2</sup> represents a saturated aliphatic C<sub>1</sub> hydrocarbon group, R<sup>2</sup> is substituted with at least one substituent.

3. The vitamin D derivative of claim 1 which is represented by Formula (III):



wherein

$R^1$  represents a saturated aliphatic  $C_{1-15}$  hydrocarbon group optionally substituted with 1 to 3 hydroxy or protected hydroxy groups; and

$R^2$  represents a saturated aliphatic  $C_{1-10}$  hydrocarbon group optionally substituted with one or more substituents, which may be the same or different and which are selected from the group consisting of a hydroxy group, a halogen atom, a cyano group, a lower alkoxy group, an amino group and an acylamino group, provided that when  $R^2$  represents a saturated aliphatic  $C_1$  hydrocarbon group,  $R^2$  is substituted with at least one substituent.

4. The vitamin D derivative according to one of claims 1

to 3, wherein R<sup>2</sup> is a hydroxymethyl group, a hydroxyethyl group, a hydroxypropyl group, a hydroxybutyl group, a hydroxypentyl group, a hydroxyhexyl group, an ethyl group, a propyl group, a butyl group, a pentyl group or a hexyl group.

5. The vitamin D derivative according to one of claims 1 to 4, wherein R<sup>1</sup> is a 4-hydroxy-4-methylpentyl group.

6. The vitamin D derivative according to claim 1 selected from the group consisting of

(5Z,7E)-(1S,2S,3R,20R)-9,10-seco-5,7,10(19)-cholestatriene-2-hydroxymethyl-1,3,25-triol,

(5Z,7E)-(1S,2S,3R,20R)-9,10-seco-5,7,10(19)-cholestatriene-2-(2'-hydroxyethyl)-1,3,25-triol,

(5Z,7E)-(1S,2S,3R,20R)-9,10-seco-5,7,10(19)-cholestatriene-2-(3'-hydroxypropyl)-1,3,25-triol,

(5Z,7E)-(1S,2S,3R,20R)-9,10-seco-5,7,10(19)-cholestatriene-2-(4'-hydroxybutyl)-1,3,25-triol,

(5Z,7E)-(1S,2S,3R,20R)-9,10-seco-5,7,10(19)-cholestatriene-2-(5'-hydroxypentyl)-1,3,25-triol,

(5Z,7E)-(1S,2S,3R,20R)-9,10-seco-5,7,10(19)-cholestatriene-2-(6'-hydroxyhexyl)-1,3,25-triol,

(5Z,7E)-(1S,2S,3R,20R)-9,10-seco-5,7,10(19)-cholestatriene-2-ethyl-1,3,25-triol,

(5Z,7E)-(1S,2S,3R,20R)-9,10-seco-5,7,10(19)-cholestatriene-2-propyl-1,3,25-triol,

(5Z,7E)-(1S,2S,3R,20R)-9,10-seco-5,7,10(19)-cholestatriene-2-butyl-1,3,25-triol,

(5Z,7E)-(1S,2S,3R,20R)-9,10-seco-5,7,10(19)-cholestatriene-

2-pentyl-1,3,25-triol and

(5Z,7E)-(1S,2S,3R,20R)-9,10-seco-5,7,10(19)-cholestatriene-  
2-hexyl-1,3,25-triol.

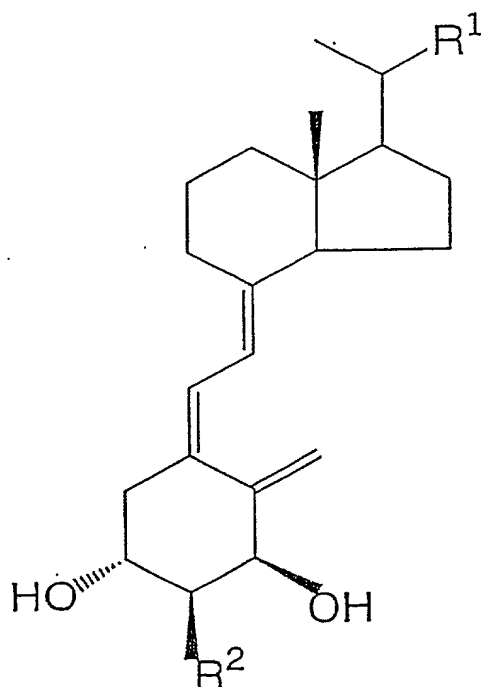
7. A pharmaceutical composition comprising a vitamin D derivative according to any one of claims 1 to 6 as an active ingredient.

8. The pharmaceutical composition according to claim 7, wherein the composition is a therapeutic agent for a disease associated with abnormal calcium metabolism, an antitumor agent or an immunomodulator.

ABSTRACT

An object of the present invention is to synthesize a novel vitamin D<sub>3</sub> derivative having a substituent at the 2 $\alpha$ -position.

5        The present invention provides vitamin D derivatives represented by Formula (I):



(I)

wherein

R<sup>1</sup> represents a saturated aliphatic C<sub>1-15</sub> hydrocarbon group optionally substituted with 1 to 3 hydroxy or protected  
10 hydroxy groups; and  
R<sup>2</sup> represents a saturated aliphatic C<sub>1-10</sub> hydrocarbon group optionally substituted with one or more substituents, which may be the same or different and which are selected from the group consisting of a hydroxy group, a halogen atom, a

cyano group, a lower alkoxy group, an amino group and an acylamino group, provided that when  $R^2$  represents a saturated aliphatic  $C_1$ hydrocarbon group,  $R^2$  is substituted with at least one substituent.



## Combined Declaration for Patent Application and Power of Attorney

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; and that I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

2α-SUBSTITUTED VITAMIN D DERIVATIVES

the specification of which (check one)

- ☐ is attached hereto;  
☐ was filed in the United States under 35 U.S.C. §111 on \_\_\_\_\_, as  
 U.S. Appl. No. \_\_\_\_\_\*; or  
☒ was/will be filed in the U.S. under 35 U.S.C. §371 by entry into the U.S. national stage of an international (PCT) application, PCT/JP00/05743 filed Aug. 25, 2000, entry requested on \_\_\_\_\_\*; national stage application received U.S. Appl. No. \_\_\_\_\_\*, §371/§102(e) date \_\_\_\_\_\* (\* if known)

and was amended on \_\_\_\_\_ (if applicable).

(include dates of amendments under PCT Art. 19 and 34 if PCT)

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above; and I acknowledge the duty to disclose to the Patent and Trademark Office (PTO) all information known by me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §§ 119 and 365 of any prior foreign application(s) for patent or inventor's certificate, or prior PCT application(s) designating a country other than the U.S., listed below with the "Yes" box checked and have also identified below any such application having a filing date before that of the application on which priority is claimed:

<u>241650/1999</u>	<u>Japan</u>	<u>27/8/1999</u>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
(Number)	(Country)	(Day Month Year Filed)	YES	NO
<u>                    </u>	<u>                    </u>	<u>                    </u>	<input type="checkbox"/>	<input type="checkbox"/>
(Number)	(Country)	(Day Month Year Filed)	YES	NO

I hereby claim the benefit under 35 U.S.C. §120 of any prior U.S. non-provisional application(s) or prior PCT application(s) designating the U.S. listed below, or under §119(e) of any prior U.S. provisional applications listed below, and, insofar as the subject matter of each of the claims of this application is not disclosed in such U.S. or PCT application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the PTO all information as defined in 37 C.F.R. §1.56(a) which occurred between the filing date of the prior application and the national filing date of this application:

<u>                    </u>	<u>                    </u>	<u>                    </u>
(Application No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)
<u>                    </u>	<u>                    </u>	<u>                    </u>
(Application No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)
<u>                    </u>	<u>                    </u>	<u>                    </u>
(Application No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)

As a named inventor, I hereby appoint the following registered practitioners to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

### All of the practitioners associated with Customer Number 001444

Direct all correspondence to the address associated with Customer Number 001444; i.e.,

**BROWDY AND NEIMARK, P.L.L.C.**  
**624 Ninth Street, N.W.**  
**Washington, D.C. 20001-5303**  
**(202) 628-5197**

The undersigned hereby authorizes the U.S. Attorneys or Agents appointed herein to accept and follow instructions from YUASA AND HARA as to any action to be taken in the U.S. Patent and Trademark Office regarding this application without direct communication between the U.S. Attorneys or Agents and the undersigned. In the event of a change of the persons from whom instructions may be taken, the U.S. Attorneys or Agents appointed herein will be so notified by the undersigned.

Title: 2 $\alpha$ -SUBSTITUTED VITAMIN D DERIVATIVESU.S. Application filed \_\_\_\_\_, Serial No. \_\_\_\_\_  
PCT Application filed August 25, 2000, Serial No. PCT/JP00/05743

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FULL NAME OF FIRST INVENTOR Hiroaki TAKAYAMA		INVENTOR'S SIGNATURE <i>Hiroaki Takayama</i>	DATE Oct. 31, 2001
RESIDENT Tokyo, Japan JPX		CITIZENSHIP Japanese	
POST OFFICE ADDRESS 2-6-12, Hatagaya, Shibuya-ku, Tokyo 151-0072 Japan			
FULL NAME OF SECOND JOINT INVENTOR Toshie FUJISHIMA		INVENTOR'S SIGNATURE <i>Toshie Fujishima</i>	DATE Oct. 31, 2001
RESIDENT Tokyo, Japan JPX		CITIZENSHIP Japanese	
POST OFFICE ADDRESS 321-2-201, Higashiasakawa-machi, Hachioji-shi, Tokyo 193-0834 Japan			
FULL NAME OF THIRD JOINT INVENTOR Yoshitomo SUHARA		INVENTOR'S SIGNATURE <i>Yoshitomo Suhara</i>	DATE Oct. 31, 2001
RESIDENT Kanagawa, Japan JPX		CITIZENSHIP Japanese	
POST OFFICE ADDRESS 5-7-301, 1258, Tokaichiba-cho, Midori-ku, Yokohama-shi, Kanagawa 226-0025 Japan			
FULL NAME OF FOURTH JOINT INVENTOR Ken-ichi NIHEI		INVENTOR'S SIGNATURE <i>Kenichi Nihai</i>	DATE Oct. 31, 2001
RESIDENT Kanagawa, Japan JPX		CITIZENSHIP Japanese	
POST OFFICE ADDRESS 3-5-12-A103, Kubosawa, Shiroyama-machi, Tsukui-gun, Kanagawa 220-0105 Japan			
FULL NAME OF FIFTH JOINT INVENTOR Katsuhiro KONNO		INVENTOR'S SIGNATURE <i>Katsuhiro Konno</i>	DATE Oct. 31, 2001
RESIDENT Kanagawa, Japan JPX		CITIZENSHIP Japanese	
POST OFFICE ADDRESS 1245-18, Chigira, Sagamiko-machi, Tsukui-gun, Kanagawa 199-0104 Japan			
FULL NAME OF SIXTH JOINT INVENTOR		INVENTOR'S SIGNATURE	DATE
RESIDENT		CITIZENSHIP	
POST OFFICE ADDRESS			
FULL NAME OF SEVENTH JOINT INVENTOR		INVENTOR'S SIGNATURE	DATE
RESIDENT		CITIZENSHIP	
POST OFFICE ADDRESS			

ALL INVENTORS MUST REVIEW APPLICATION AND DECLARATION BEFORE SIGNING. ALL ALTERATIONS MUST BE INITIALED AND DATED BY ALL INVENTORS PRIOR TO EXECUTION. NO ALTERATIONS CAN BE MADE AFTER THE DECLARATION IS SIGNED. ALL PAGES OF DECLARATION MUST BE SEEN BY ALL INVENTORS.